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UNIVERSITY OF ALBERTA

IMMUNOMODULATION OF INTESTINAL TRANSPLANTS USING RELATED DONORS

BY

MARTIN SHELDON FRIEDLICH

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science.

IN

EXPERIMENTAL SURGERY

DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

Fall 1995

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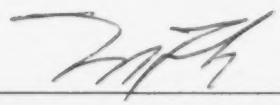

Martin Sheldon Friedlich
7508 - 152 Street
Edmonton, Alberta
T5R 1K5

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UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled IMMUNOMODULATION OF INTESTINAL TRANSPLANTS USING RELATED DONORS submitted by MARTIN FRIEDLICH in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in EXPERIMENTAL SURGERY

Dr. D. Sigalet (Supervisor)

Dr. N. Kneteman

Dr. G. Warnock

Dr. R. Fedorak

May 8, 1995

DEDICATION

I would like to dedicate this thesis to my father,
Dr. Thomas Friedlich

Abstract

In order to study the effect of partial matching on graft protection and function we have developed a pig model of living related small intestinal transplantation (SIT). Littermate outbred pigs weighing 10-15 kg underwent Swine Leukocyte Antigen (SLA) typing of their DR locus by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism Analysis. Controls (n=5) underwent resection of all jejunum and ileum except the distal 150 cm. Continuity was restored by anastomosing duodenum to ileum. The transplant group (n=20) was divided into SLA matched (n=8), haploidentical (n=4), and SLA mismatched (n=8). A two stage procedure was planned. The allograft consisted of the distal 150 cm of ileum with portocaval drainage and initial heterotopic placement. Two weeks later the native small intestine was resected, and the graft placed orthotopically. Immunosuppression for all groups consisted of methylprednisolone 1mg/kg for 3 days, and cyclosporine IV followed by oral adjusted to maintain a target level of 250-350 ng/mL. All animals were followed for a six week study period and then intestinal permeability assessed by measuring urinary recovery of orally administered ^{99}Technetium-diethylenetriamine pentacetic acid (^{99}Tc-DTPA). Rejection and Graft Versus Host Disease (GVHD) were assessed histologically. All animals tolerated the procedure well with a relatively low (15%) technical failure rate. All Transplant animals were able to maintain the target cyclosporine level. In the control group, one animal died from a bowel obstruction at 21 days post-op. Surviving animals (n=4) attained a weight gain of 20.19 ± 20.54% of the starting weight. Intestinal permeability was 0.18± 0.08% of total dose administered. In the matched group, one animal died from a technical complication (bowel obstruction) at 7 days post - transplant, three animals were euthanized at 10, 24 and 27 days post-transplant for histological evidence of rejection, and one animal was euthanized at day 9 for histological evidence of GVHD. Surviving animals in this group (n=3), attained a weight gain of 12.06%, with intestinal permeability of 0.15±0.12%. In the haploidentical group , animals survived a mean of 15

days. One animal died from a technical complication at day 13. Two animals showed signs of GVHD (dermatitis, diarrhea, lymphadenopathy), and one animal developed severe rejection. These findings were confirmed histologically. In the mismatched group one animal died from a technical complication (venous thrombosis), at day 2. Four animals were euthanized for histological evidence of rejection at days 13, 14 (n=2) and 24 . Surviving animals (n=3) in this group attained a weight gain of 15.26%, with intestinal permeability of 3.22±2.68% which was significantly higher than the matched group ($p<0.05$). We conclude that transplantation of the distal ileum provides adequate absorptive surface for weight gain and cyclosporine absorption. Intestinal permeability is increased in SLA mismatched animals which may be an early sign of rejection. Unexpectedly, some partially matched grafts develop a severe form of GVHD which culminates in the death of the animal. These findings may have implications for clinical attempts at SIT.

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LIST OF ABBREVIATIONS

^{99}Tc-DTPA	^{99}Technitium-diethylenetriamine pentacetic acid
APC	Antigen Presenting Cell
ATG	Anti Thymocyte Globulin
DLA	Dog Leukocyte Antigen
DNA	Deoxyribose Nucleic Acid
GALT	Gut-Associated Lymphoid Tissue
GI	Gastrointestinal
GVH	Graft versus Host
GVHD	Graft versus Host Disease
HLA	Human Leukocyte Antigen
HPLC	High Performance Liquid Chromatography
IVC	Inferior Vena Cava
MHC	Major Histocompatibility Complex
MLC	Mixed Lymphocyte Cultures
PCR-RFLP	Polymerase Chain Reaction - Restriction Fragment Length Polymorphism
RIA	Radioimmunoassay
RT-PCR	Reverse Transcription - Polymerase Chain Reaction
SBT	Small Bowel Transplant
SIT	Small Intestinal Transplantation
SLA	Swine Leukocyte Antigen
SMA	Superior Mesenteric Artery
SMV	Superior Mesenteric Vein
TPN	Total Parenteral Nutrition
WBC	White Blood Cell Count

INTRODUCTION

Short bowel syndrome occurs when there is loss of sufficient small bowel to prevent adequate absorption of nutrients. Its etiology in children includes: necrotizing enterocolitis, volvulus and strangulated abdominal wall defects.[1] In adults it is most commonly caused by mesenteric vascular disease, inflammatory bowel disease and trauma.[2] When short bowel syndrome occurs, and is severe enough that the patient cannot survive on enteral nutrition alone, then TPN is used to maintain nutritional requirements. TPN is an expensive treatment, costing $20,000-70,000/yr.[3,4] In addition, TPN use is associated with complications mostly related to venous access, including difficulty in establishing venous access as well as catheter related sepsis.[5] TPN has also been associated with liver injury.[6] Small intestinal transplantation (SIT) has been proposed as a method for treating short bowel syndrome. Unfortunately it has proven to be one of the most difficult organs to transplant due to its increased immunogenicity resulting from its large amount of lymphoid tissue. This increased immunogenicity manifests as either a Host versus Graft reaction or as a Graft versus Host reaction. This organ is unique because rejection not only leads to loss of function but also to bacterial translocation. The risk of severe sepsis is increased further by the addition of immunosuppressive therapy.[7]

Research in the field of small intestinal transplantation in the 1960's and 1970's focused on the development of the surgical technique as well as initial attempts at immunomodulation of this procedure, with little success with regard to long term survival. In the 1980's the techniques were refined and with the development of cyclosporine, long term survival now became possible in large animals models of SIT.[8,9,10,11] With the success of this procedure in large animal models particularly in the porcine model, researchers began clinical SIT programs.[12,13] Unfortunately, investigators in the early 1990's are realizing that the same immunosuppression that is responsible for long term survival carries with it the added risk of infections[14,15] and

lymphoproliferative disease.[16,17] The task for researchers during this decade will be to provide some form of immunomodulation to this transplantation procedure so as to be able to achieve long term survival with minimal risk of fatal complications.

Living related transplantation has proven to be beneficial in prolonging kidney allograft survival.[18,19,20] It is conceivable that by applying the same form of immunomodulation to SIT, long term survival with minimal immunosuppression would be attainable. To explore this area in more detail a new animal model is required which would resemble the human situation as closely as possible.

Large animal models of SIT have been plagued with high technical failure rates in the past.[9,11] In addition there have been no reports of a model of living related SIT. It would therefore be important to establish a new model of SIT that is reproducible with a low technical failure rate, and that can be applied to living related SIT.

The pig is the ideal study animal for SIT as it resembles humans anatomically, physiologically,[21] and immunologically.[22] Its Swine Leukocyte Antigen (SLA) system, has been shown to be homologous to its human counterpart (HLA).[23,24] With recent technological advances, rapid typing of the SLA locus is possible.[25,26] This makes it possible to MHC type prospective donors and recipients of porcine SIT. This form of immunomodulation combined with a new surgical technique should result in decreased immunogenicity of the graft, and thus make successful SIT possible utilizing an immunosuppressive strategy that would be tolerable in humans .

This thesis is presented in paper format. The development of the animal model is divided into surgical technique, and SLA typing which are discussed separately in chapters 2 and 3 respectively. The application of this animal model in the immunomodulation of intestinal transplants using related donors is examined in chapter 4.

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SURGICAL TECHNIQUE

Introduction

Large animal models of small intestinal transplantation (SIT) have been plagued with high technical failure rates in the past.[1,2] In addition there have been no reports of a model for living related SIT. It was for these reasons that we felt it was important to establish a new model of SIT that was reproducible with a low technical failure rate, and that could be applied to living related SIT.

Orthotopic vs. Heterotopic Small Intestinal Transplantation

Two types of surgical techniques have become popular in the field of small intestinal transplantation. The first, orthotopic transplantation occurs when the small intestine is brought into continuity with the remaining native small bowel, after the majority of the native small intestine has been resected. Heterotopic SIT describes a technique where the small bowel is hooked up to its vascular supply, however, it is not brought into continuity with the native bowel. In these cases, one or both ends of the allograft are brought out to the skin surface as a stoma, while the native bowel is left to function as it did in its pre-operative state. There are advantages and disadvantages to each of these surgical techniques. With the orthotopic technique, one is able to study the survival as well as the function of the small bowel that is transplanted. The disadvantage of this technique is that it requires immediate function of the graft in all aspects in order for the recipient to survive. The advantages and disadvantages of the heterotopic procedure are just the opposite of the orthotopic - that is with this procedure all the confounding problems of hooking this segment of bowel up in continuity are abolished, but in return one is not able to study the function of the allograft, one is only able to study the survival of the allograft, and the immunological response which results from the transplantation. Both of these techniques are described in the literature in the rat model,[3,4] as well as in the canine,[1,5,6] and porcine models.[7,8,9] In 1971, Monchik and Russell introduced a microsurgical procedure for the

transplantation of the entire small bowel of the rat as an auxiliary heterotopic graft.[3] They used this technique to perform small bowel transplantation for the first time between animals with well characterized genetic backgrounds. In 1973, Kort et. al. described orthotopic total small bowel transplantation in the rat in which the native small bowel was resected and the donor small bowel was anastomosed to the duodenum of the host proximally and distally to the cecum.[4] This technique would go on to provide a mechanism for studying the response of the host as well as the graft to transplantation, but in addition it would provide a method to study the function of small bowel allografts. Early researchers studying canine small intestinal transplantation, transplanted segments of donor intestine heterotopically into the recipient's neck.[5] This location was chosen, for ease of access to a vascular supply, as well as ease of monitoring the segments of bowel for signs of rejection. The orthotopic small bowel transplant in the canine model has been reported extensively in the literature.[1,6] Lillehei used this model to study the survival of allografts as early as 1959.[10,11] Later in the 1980's this model was used to evaluate the interaction of allograft rejection and Graft Versus Host Disease (GVHD) as well as to study the functional capabilities of the graft such as nutrient and immunosuppressant absorption.[1,6] In the porcine model, both heterotopic and orthotopic SIT have been performed. Kaneko et. al. in 1989 used both the heterotopic and orthotopic model to show that small bowel transplantation can be achieved with adequate use of immunosuppression in the porcine model.[7] Other researchers have used the orthotopic technique to study survival of small intestinal allografts, as well as growth, nutrient absorption, and immunosuppressant absorption.[2,12]

Portal vs. Caval Drainage

The most appropriate venous drainage for a small intestinal allograft has remained a controversial issue in the literature.[13] The advocates of portal - portal drainage claim that in addition to the potential immunological benefits, drainage of the allograft via the portal route establishes normal physiological conditions and avoids potential metabolic

alterations that follow portal systemic shunting.[14] This same group showed however, that even though portal caval anastomosis is followed by alterations (hyperammonemia, a trend toward liver atrophy) that are similar to those seen after total portal caval shunts, these alterations are much less pronounced and they had no clinically observable consequences in the rat model.[15] Other researchers found no advantage in graft survival, nutritional state, or liver function with either portal-portal or portal-caval drainage.[16] Hale et. al. experimenting with SIT in non-human primates showed that systemic venous drainage of the graft did not cause any significant metabolic abnormalities.[17] The main reason that systemic venous drainage may be preferable to portal venous drainage in SIT is because it would be technically easier and safer to perform in the clinical setting.[7]

Total vs. Segmental Intestinal Transplantation

One would think that when transplanting small intestine, it would be advantageous to utilize as much small intestine as possible, as this would provide the recipient with as much absorptive surface area as possible. Most SITs in animal models[1,6,12,23], as well as clinical intestinal transplantation[18,19,20] have utilized the entire length of small bowel for transplantation. It may however, be more realistic to perform a segmental intestinal transplant. Ruiz and Lillehei were the first to propose this when in 1972 they stated, " when intestinal allotransplantation is used to maintain nutrition in man, there would seem to be no need to use the entire small intestine of a cadaver; one or two metres should be sufficient".[11] Using a segment of small intestine would offer certain advantages over the use of the entire small bowel. Firstly, it would be much easier to fit a segment of small bowel into the abdomen of a recipient which probably has spatial constraints from scarring due to previous abdominal surgeries. Secondly, segmental intestinal transplants offer some immunological advantages, as there would be less gut associated lymphoid tissue in the smaller piece of bowel, and therefore would decrease the risk of rejection and GVHD.[22] The third advantage of using the segmental small bowel allograft, is that this might allow for transplantation from

a living related donor. [8] As far as which segment of bowel to use as a graft, the ileum has a large functional reserve and is capable of providing adequate absorption of carbohydrate, protein and fat, as well as being able to absorb bile acids and vitamin B12. [21] The ileum however, does contain a large amount of lymphoid tissue which may confer to it a greater immunogenicity, thereby inducing a stronger rejection reaction , as well as increasing the risk of a potential GVH reaction.[22]

We describe the development of a two stage orthotopic segmental SIT. The development of this technique progressed through a series of control animals that were necessary prerequisites to the transplant model, and in addition served as a comparison for weight gain and cyclosporine absorption in the transplant groups.

Methods

Outbred littermate pigs weighing 10-15 kg underwent Swine Leukocyte Antigen (SLA) typing of their DR locus by Polymerase Chain Reaction- Restriction Fragment Length Polymorphism analysis (PCR-RFLP). Animals were then selected as donor- recipient pairs as follows: SLA matched (M) (n=4), Haploidentical (H) (n=3), and SLA mismatched (MM) (n=5). All animals underwent a two-stage orthotopic segmental ileal intestinal transplant. The allograft consisted of the distal 150 cm of ileum. The allograft was initially placed heterotopically, with portocaval drainage. Two weeks later, the native bowel was resected, and the allograft placed orthotopically. Animals were followed for a six week study period.

Transplantation of Small Intestine

Donor Operation

Animals were premedicated with intramuscular ketamine and atropine, and were then anesthetized with 4% halothane, and maintained on 1% halothane for the duration of the case. A right external jugular line was placed for venous access. A laparotomy was performed. The spiral colon was identified, and the peritoneal attachments taken down so as to expose the mesentery of the colon. A point on the transverse colon immediately exiting the spiral colon was transected, following which the mesentery of the colon was divided to a point 10 cm proximal to the ileocecal valve. The ileum was divided with a GIA 50 stapler (autosuture), and the colon was then discarded (figure 2.1). A pre-measured umbilical tape was used to measure a point 160 cm proximal to the distal resection margin of the ileum. The ileum at this point was stapled using a GIA 50 stapler. This 160 cm of ileum would become the allograft. The remaining ileum and jejunum were resected staying peripheral in the mesenteric arcade at the level of the vasa recta. In the pig, the duodenum and large bowel wrap around each other at the level of the pancreas, and both of these structures also wrap around the root of the small bowel

mesentery containing the SMA and SMV. It was therefore necessary to unravel the duodenum as well as the large bowel, to gain access to the superior mesenteric vessels. The portal vein was cleared to above the level of the splenic vein (figure 2.2). The splenic vein was ligated adjacent to the portal vein, following which a splenectomy was performed to gain better access to the supraceliac aorta. The pancreas was peeled off the portal vein, and splenic vein, and then removed. The infrarenal aorta was cannulated with a 10 Fr aortic cannula (figure 2.3). The supraceliac aorta was encircled with a ligature. The celiac artery was ligated just distal to the aorta. The animal received heparin 4000 units iv and phentolamine 1 mg/kg iv. The graft was flushed in situ with 2 Litres of Ringers + 2.4% Mannitol solution. The portal vein was transected just above the splenic vein to vent the system. The allograft was removed with a large aortic cuff.

Recipient Operation

Stage I

The animal was anesthetized in the same manner as the donor. A double lumen catheter was placed in the right external jugular vein, and tunneled to the back of the animal. The animal received Cefazolin 500 mg iv, and Ranitidine 25 mg iv. A laparotomy was performed, and the small bowel and colon were reflected superiorly to visualize the infrarenal aorta and IVC. The infrarenal IVC was cleared for 4 cm, and the aorta was cleared for 3 cm using blunt dissection. This was often the most difficult part of the operation as there is a lymphatic sac that usually lies on the anterior aspect of the aorta, and must be gently pushed to the side so as not to enter it. If this sac is entered, a lymphatic leak will result, and unless it is closed intraoperatively, the animal will develop postoperative ascites. Other researchers have described similar concerns with this lymphatic sac.[23] The animal received heparin 100mg/kg iv, and then proximal and distal vascular clamps were placed on the IVC (figure 2.4). A large venotomy was made in the IVC, and the donor portal vein anastomosed end-to-side using a running stitch of 6-0

polypropylene. Following completion of the venous anastomosis a bulldog clamp was placed on the donor portal vein while the vascular clamps on the native IVC were removed. We found it more effective to use a Satinsky clamp on the aorta rather than two vascular clamps as this was a safer way of dealing with the lymphatic sac. An arteriotomy was made, and then the superior part of the donor aortic cuff was anastomosed end-to-side to the recipient aorta using a running stitch of 6-0 polypropylene. Prior to releasing the Satinsky and bulldog clamps, the animal was given sodium bicarbonate 3 mEq/kg, and calcium chloride 30 mg/kg iv. The grafted bowel immediately regained its natural pink color. The distal end of the allograft was brought out through a stoma in the right lower quadrant (figure 2.5). The abdomen was closed, and the stoma matured. The animal received 1 litre of Ringers Lactate solution throughout the operation.

Stage II (performed two weeks following stage I)

The animal was anesthetized in the same manner as in stage I. A new external jugular line was placed on the left side, and tunneled to the back. The laparotomy incision was reopened, and the native bowel and allograft were dissected free from one another as there were usually many adhesions. The native bowel was marked off 10 cm proximal to the ileocecal valve, then all of the ileum and jejunum were resected proximally. The proximal end of the allograft was anastomosed to the duodenum. The distal end of the allograft was left as a stoma, but at 10 cm proximal to the stoma, the allograft was divided using a GIA 50 stapler, and the new distal end was anastomosed to the remaining native ileum. The original distal end of the allograft became a Thiry Vella Fistula (figure 2.6). All anastomoses were constructed using 4-0 silk in interrupted stitches. The abdomen was closed. The animal received 750 mL of Ringers Lactate solution during the procedure.

Control procedure

Animals were premedicated with intramuscular atropine and ketamine and were then anesthetized with 4% halothane until surgical anesthesia was attained at which point they were maintained on 1% halothane. A laparotomy was performed. The small bowel was identified, and a point 10cm proximal to the ileocecal valve was marked (figure 2.7). Using a pre-measured umbilical tape the small bowel was measured for 160 cm proximal to the distal marking. At this point the small bowel was divided using a GIA 50 stapler. Next the bowel proximal to this point was resected staying distal in the mesentery. This was carried out up to the point where the jejunum meets the c-loop of the duodenum, at which point the bowel was transected and discarded. Continuity was reestablished by anastomosing the ileum to the duodenum. However, first the proximal 10 cm of ileum was used to construct a Thiry vella fistula (figure 2.8). A sham transection and reanastomosis was carried out at the point marked previously 10 cm proximal to the ileocecal valve, and then the abdomen was closed, and the stoma matured.

Immunosuppression

All animals received methylprednisolone 1 mg/kg iv daily for 3 days post-op. Cyclosporine was given initially iv, and was subsequently changed on day 5 to oral cyclosporine. The initial starting dosages were 7 mg/kg/day iv, 20 mg/kg/day orally following stage I, and 30 mg/kg/day orally following stage II. Cyclosporine was dosed twice daily, and all doses were adjusted to maintain a target twelve hour whole blood trough level of 250-350 ng/mL.

Postoperative Care

All animals were housed initially in metabolic cages, and were allowed nothing by mouth for the first 48 hours. During this time they received 500 mL of iv D5 Saline + 20 mEq KCl/L twice a day. In addition they received Cefazolin 500mg iv , and Ranitidine 25 mg

iv, each twice a day. Buprenorphine 0.4 mg iv twice daily was given for analgesia. These medications were given for the first 48 hours post-op. On post-op day 3, the animals were allowed water ad lib, and were then progressed to porcine mix the following day, and then started on pig chow on post-op day 5. They were then continued on pig chow and water ad lib until the end of the study period. In addition , all animals were supplemented with an elemental form of enteral nutrition (Vivonex).

Monitoring

a) Cyclosporine Levels

Whole blood cyclosporine levels were determined using the Abbott TDX monoclonal Radioimmunoassay. Levels were determined twice weekly for the first week, and subsequently weekly.

b) Weight gain

Animals were weighed weekly. Weight gain was expressed as a percentage of the starting weight for the control animals. In the transplant group weight gain was expressed as a percentage of the preop stage II weight (i.e. reflecting the time that the allograft was in the orthotopic position)

c) General Well-Being

All animals were monitored daily for feed intake, excretions, and general well-being. Animals were observed daily for signs of GVHD (dermatitis, diarrhea, lymphadenopathy), or rejection (weight loss, diarrhea, decreased appetite, lethargy)

Determination of Study Endpoint

There were three possible ways that the study could be ended:

1. Animals that survived the 6 week study period were euthanized at the time of the final laparotomy.

2. At the time of the second stage procedure, if the allograft was determined to be nonviable by visual inspection, the animal was euthanized at that time.

3. If during any other time in the study the animal looked unwell including either weight loss > 10% of the starting weight, continued loss of appetite, persistent diarrhea, persistent vomiting, or persistent lethargy, then the animal was euthanized.

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Means between the groups (control versus SLA matched versus SLA mismatched) were compared using an ANOVA. Bonferone was used as the post-test. A p value <0.05 was considered statistically significant.

Results

Control Animals

One animal died of a technical complication (bowel obstruction) on post-op day #21. The remaining four animals (table 2.1) survived the study period. All animals in this group gained weight, with weight gains ranging from 13.86 to 107.27 % of initial starting weight. The mean cyclosporine levels ranged from 166.98 ng/mL to 281.39 ng/mL. Although the animals were followed for a 6 week period, and the weight gain determined for this time period (figure 2.9), the weight gain during the first 4 weeks is recorded as well for comparison to the transplant animals (figure 2.10). This is due to the fact that even though the transplant animals were followed for a 6 week period, the allograft was in the orthotopic position for 27.75±2.63 days.

Transplant Animals

SLA Matched (M)

In this group (table 2.2), one animal (T13) was lost due to a technical complication (closed loop bowel obstruction) at day 7. One animal in this group (T12) was euthanized at day 24 (time of second stage procedure) for histological evidence of rejection. The remaining two animals (T4, T14) survived the study period. Their grafts were in the orthotopic position for a mean of 29 days. During this time they attained a mean weight gain of 3.39±8.94%. The mean cyclosporine level maintained in this group was 279.74±71.56 ng/mL.

SLA Haploidentical (H)

Haploidentical transplants (table 2.3), survived a mean of 15 days. Two animals showed signs of GVHD (dermatitis, diarrhea, lymphadenopathy). One animal developed severe rejection. None of the animals survived long enough to undergo the second stage procedure. The mean cyclosporine level maintained in this group was 432.81±147.13 ng/mL.

SLA Mismatched (MM)

In this group (table 2.4), there was one technical death (T16) which was due to thrombosis of the venous anastomosis. Two animals (T9, T11) were euthanized due to histological evidence of rejection at days 14, and 24 respectively. The two animals (T10, T15) that survived the study period had their grafts in the orthotopic position for a mean of 26.5 days. During this time period, they attained a mean weight gain of 9.91±15.89% of the starting weight. The mean cyclosporine level maintained in this group was 339.01± 75.55 ng/mL.

Transplant vs. Control

In the transplant animals (table 2.5) that survived the study period (n=4), the allograft was in the orthotopic position for 27.75±2.63 days. During this time they attained a mean weight gain of 6.65±11.18%. The control group during the same time period attained a mean weight gain of 20.19±20.54%. All groups were able to maintain the target cyclosporine level.

Discussion

In the past most researchers have used either a heterotopic[3,5] or orthotopic[2,4,6] model to study SIT. This two stage segmental SIT that is described makes use of both techniques (heterotopic, and orthotopic). By keeping the graft in a heterotopic position immediately post transplant the animals were given a chance to recover from the major transplantation procedure without having to rely on the graft for nutrient absorption, as well as absorption of cyclosporine. The animals gained weight easily with the graft in the heterotopic position (figure 2.9), and were fully recovered by the time of the second stage procedure two weeks later. It was felt that this two week wait was beneficial as it provided the allograft with time to revitalize itself from a prolonged ileus following the transplant procedure. In addition this wait provided sufficient time for the lymphatics to start to regenerate as it has been reported that no lymphatic regeneration occurs prior to two weeks post- transplant.[12] During the second stage procedure the animals underwent a much less stressful procedure, however, the post-op care required was more intense, as this was the first time that the animal was required to survive on the allograft which was significantly shorter than its native bowel. This explains the initial drop-off in weight immediately following stage II (figure 2.9). In the end, the animals that did not succumb to immunological consequences and survived did gain weight, as well as maintain the target cyclosporine levels. For the surviving animals the allograft was in the orthotopic position for 27.75± 2.63 days. During this time they attained a mean weight gain of 6.65± 11.18%, all animals regaining their preoperative body weight. The control group during the same time period attained a mean weight gain of 20.19±20.54% (figure 2.10). Segmental jejunal allografts (25% of entire small bowel) have been reported to require 50 days to regain their preoperative body weights.[8] The superior absorptive capacity of the ileum may account for this difference

This model made use of an allograft that was 150 cm in length, which translated into 20% of the small bowel. This was felt to be the minimum that the animal could survive on.[24] The ileum was chosen due to its superior absorptive capacity[21], as well as for its distinctive vascular supply from the distal part of the SMA which would make it an ideal candidate from which to establish an appropriate vascular pedicle for a living related SIT. By examining the control animals weight gain (figure 2.10), one can see that this amount of small bowel is sufficient for the animals to gain weight. It is not surprising that the transplant animals gained less weight than the control animals, as the transplant animals underwent a much larger procedure than the control animals, and additionally had to contend with the obstacle of lymphatic regeneration that would not be occurring in the control animals. Nevertheless, the transplant animals did gain weight satisfactorily.

The two stage orthotopic segmental SIT is reproducible with a relatively low (16%) technical failure rate. The two technical failures that were seen, a bowel obstruction in one, and a venous thrombosis in another, have been reported extensively in the literature.[2,7,8,9,23,25]

This model would be useful for studying living related SIT as it makes use of a large enough segment of bowel from which to absorb sufficient nutrients to gain weight, as well as sufficient cyclosporine to maintain therapeutic levels. In addition this segment is small enough for a donor to survive without. This particular model makes use of a vascular supply that involves the entire SMA and a cuff of aorta. Surely this would not be applicable to a clinical living related SIT as these structures are of vital importance. In order to circumvent this problem, the next step would be to use a more distal vascular supply from a branch of the SMA within the mesentery of the small bowel. The technique that is described here was accomplished using loupe magnification(2.5x), however, for more distal vessels a microsurgical technique would be required.

Table 2.1 Results - Control Animals

Animal I.D.	Wt. Gain (4 weeks) (%)	Wt. Gain (6 weeks) (%)	CsA level (ng/mL)
C8	-9.90	13.86	281.39
C9	36.36	27.27	203.57
C10	28.18	107.27	182.00
C13	26.13	52.25	166.98

Table 2.2 Results - SLA Matched Transplants (M)

Animal I.D.	Technical Loss	GVHD	Rejection	CsA level (ng/mL)	Survival (days)	Wt. Gain (%)
T4	no	no	no	338.98	40	9.71
T12	no	no	yes	200.23	24	N/A
T13	yes	no	no	N/A	7	N/A
T14	no	no	no	300.02	40	-2.94

Table 2.3 Results - SLA Haploidentical Transplants (H)

Animal I.D.	Technical Loss	GVHD	Rejection	CsA level (ng/mL)	Survival (days)	Wt. Gain (%)
T5	no	yes	no	296.94	17	5.63 *
T6	no	yes	no	412.43	14	-9.48 *
T7	no	no	yes	589.06	14	3.45% *

* bowel in heterotopic position, no second stage

Table 2.4 Results - SLA Mismatched Transplants

Animal I.D	Technical Loss	GVHD	Rejection	CsA level (ng/mL)	Survival (days)	Wt. Gain (%)
T9	no	no	yes	342.75	14	N/A
T10	no	no	no	259.11	40	17.78
T11	no	no	yes	288.08	24	N/A
T15	no	no	no	439.63	40	-1.32
T16	yes	N/A	N/A	N/A	2	N/A

Table 2.5 Results - Transplants versus Control Animals

Group	n	Technical Loss	GVHD	Rejection	CsA level (ng/mL)	Survival (n)	Wt. Gain (%)
Control	5	1	0	0	208.49±50.87	4	20.19±20,54
M	4	1	0	1	279.74±71.56	2	3.39±8.94
H	3	0	2	1	432.81±147.13	0	N/A
MM	5	1	0	2	339.01±75.55	2	9.91±15.89

Data mean ± S.D.

Figure 2.1 **Donor Operation**

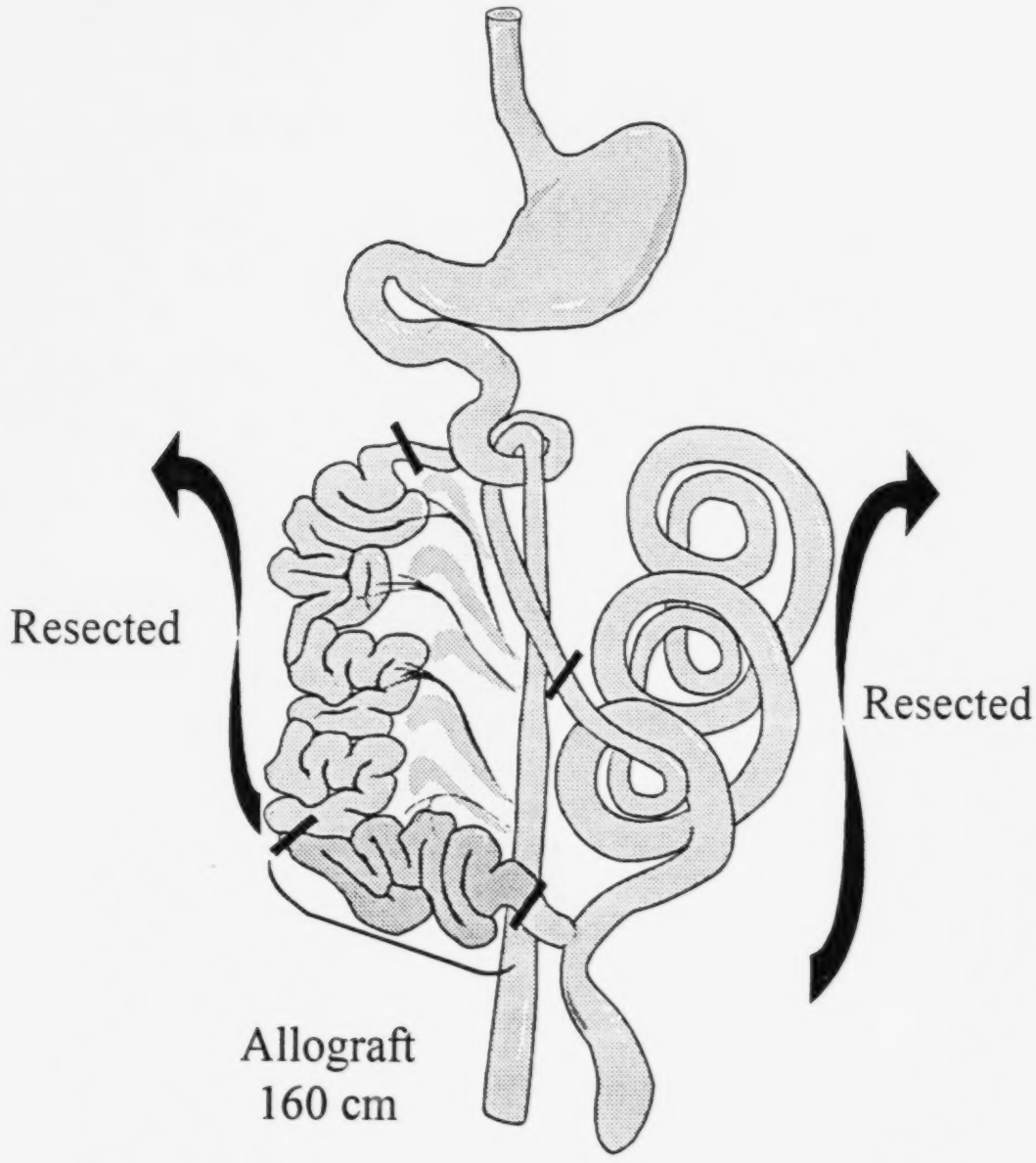


Figure 2.2 **Donor Operation**

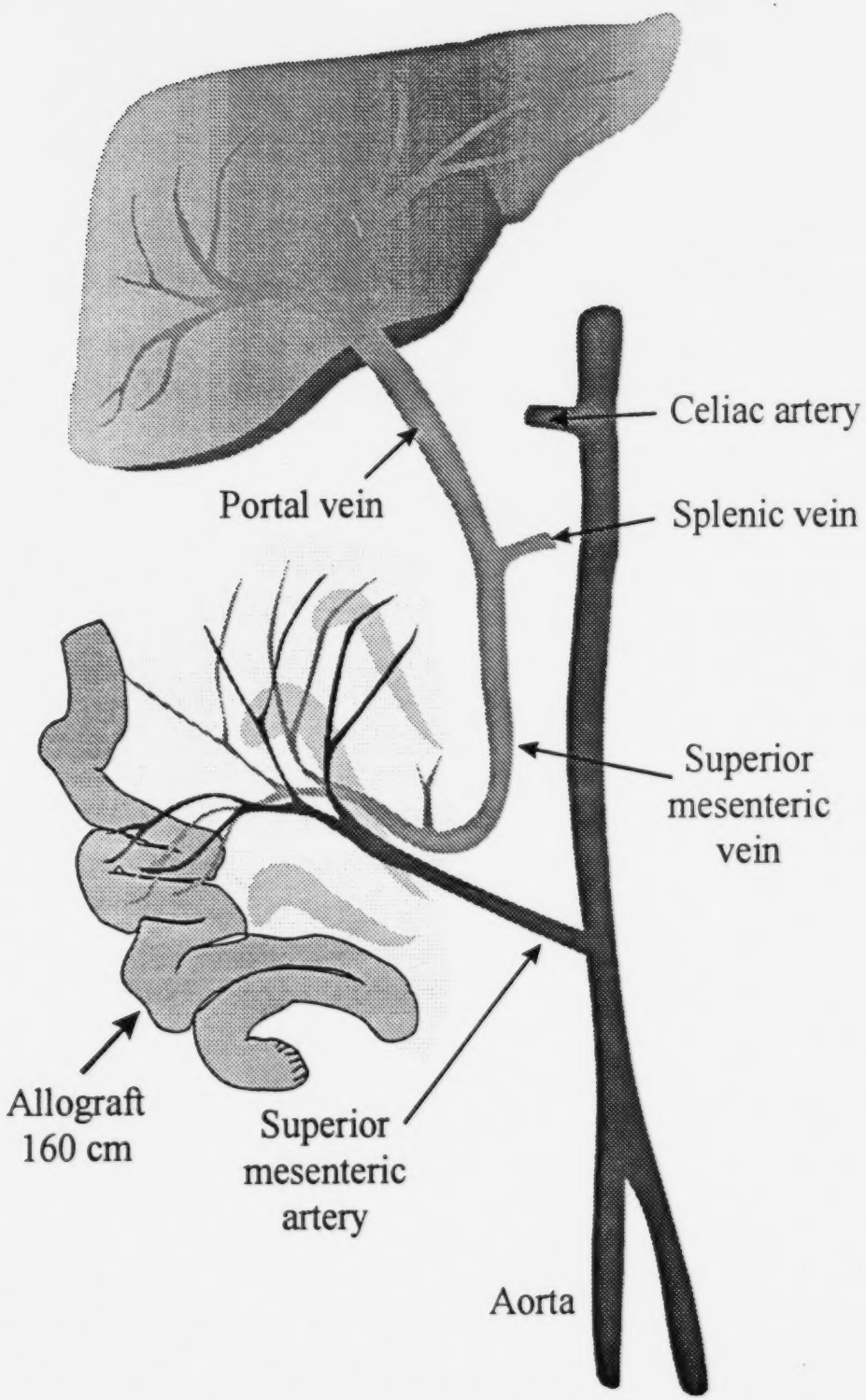


Figure 2.3 **Donor Operation**

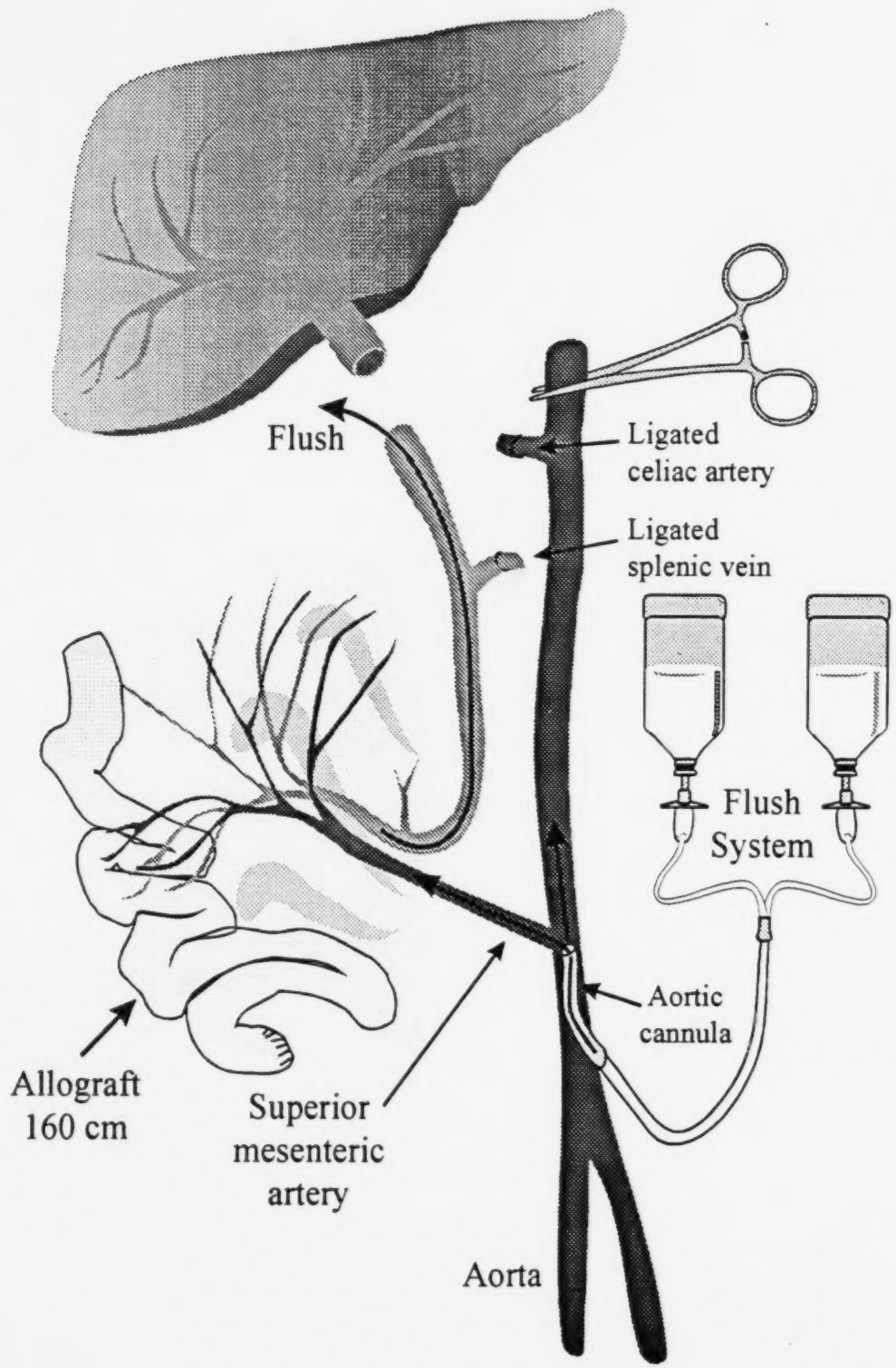


Figure 2.4 **Recipient Operation**

Stage I

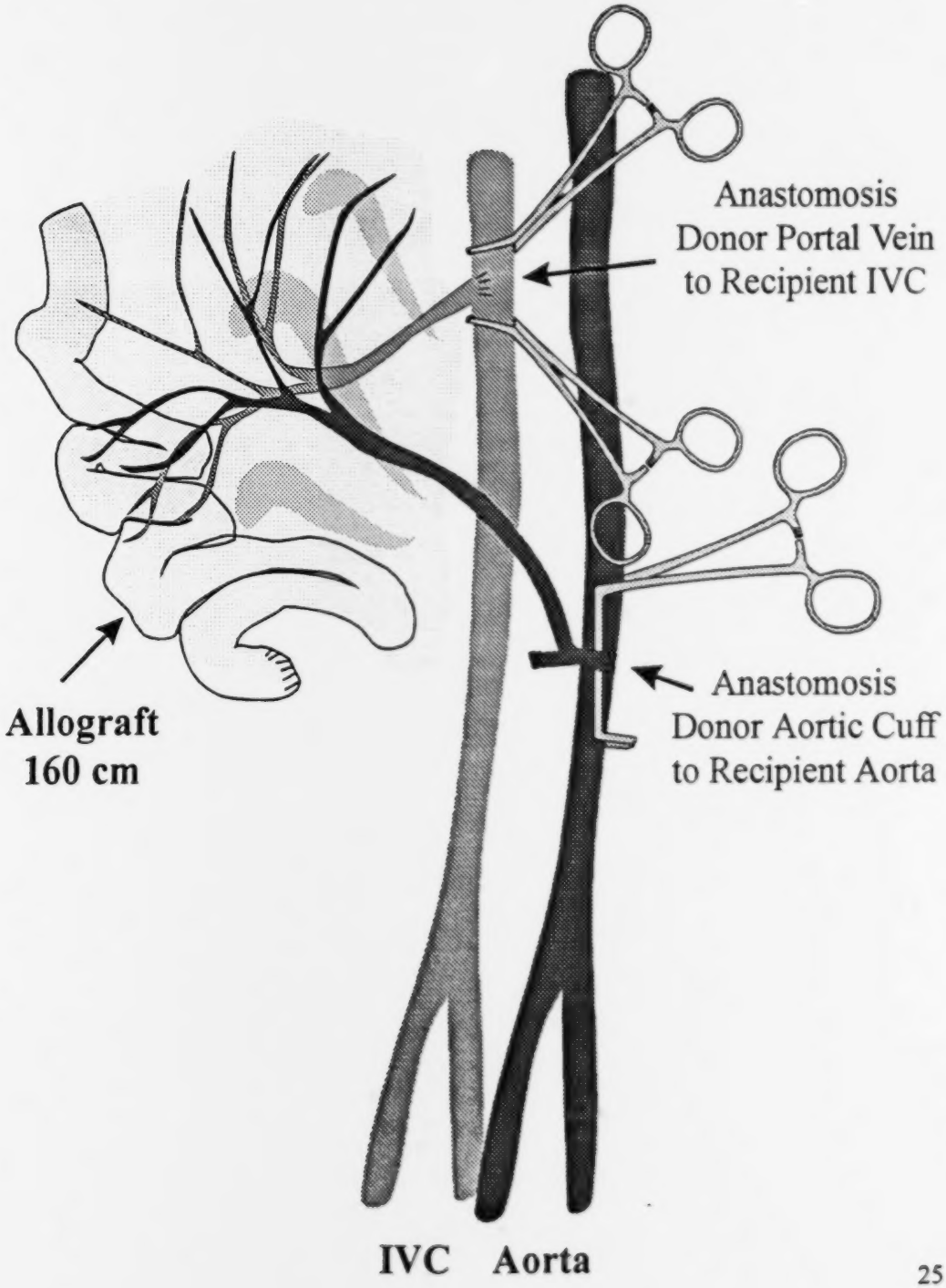


Figure 2.5 **Recipient Operation Stage I**

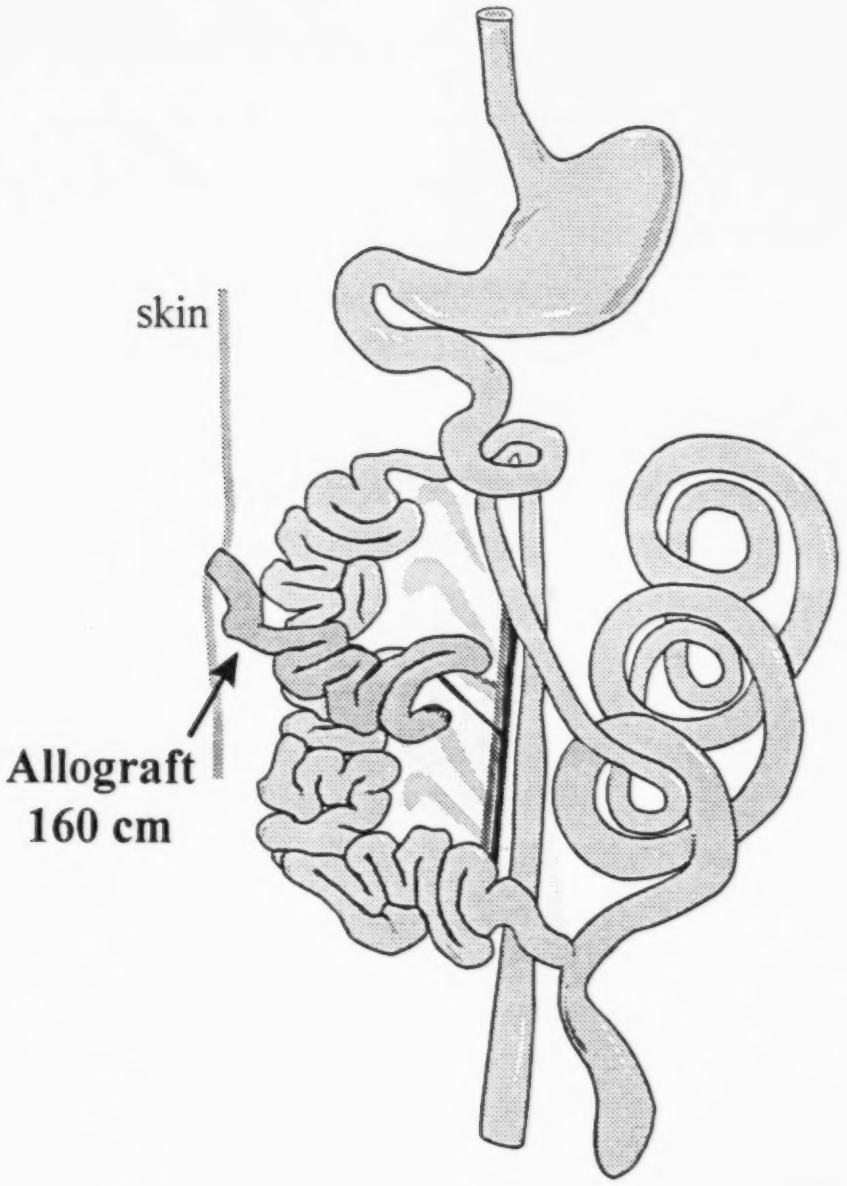


Figure 2.6 **Recipient Operation Stage II**

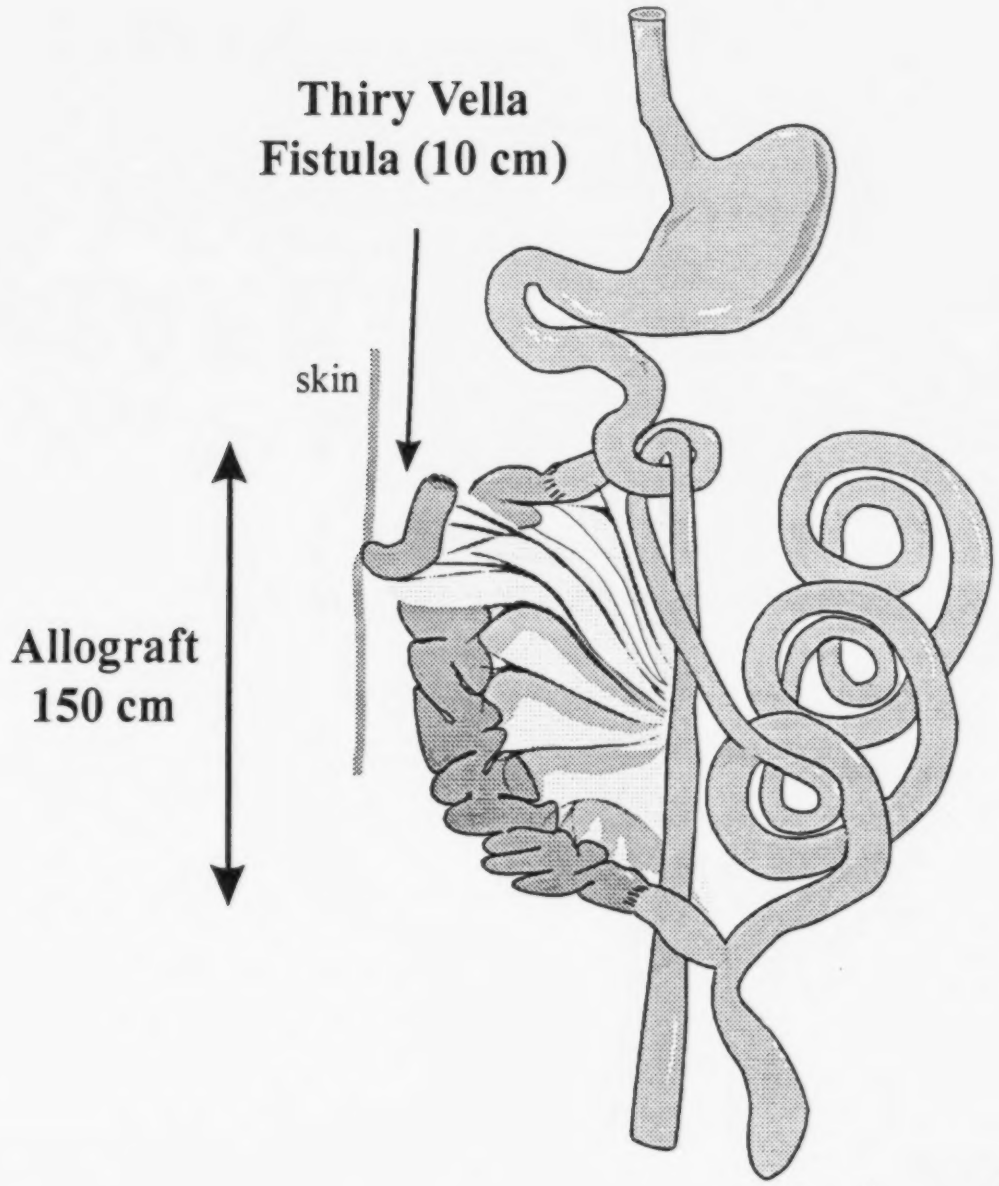


Figure 2.7 **Control Procedure**

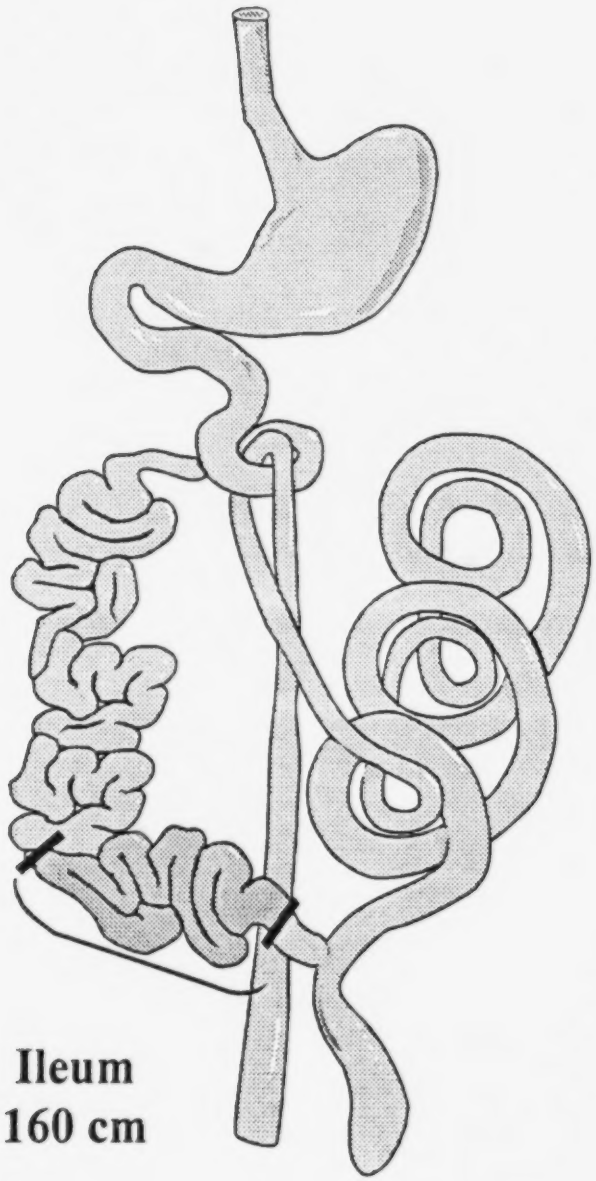


Figure 2.8 **Control Procedure**

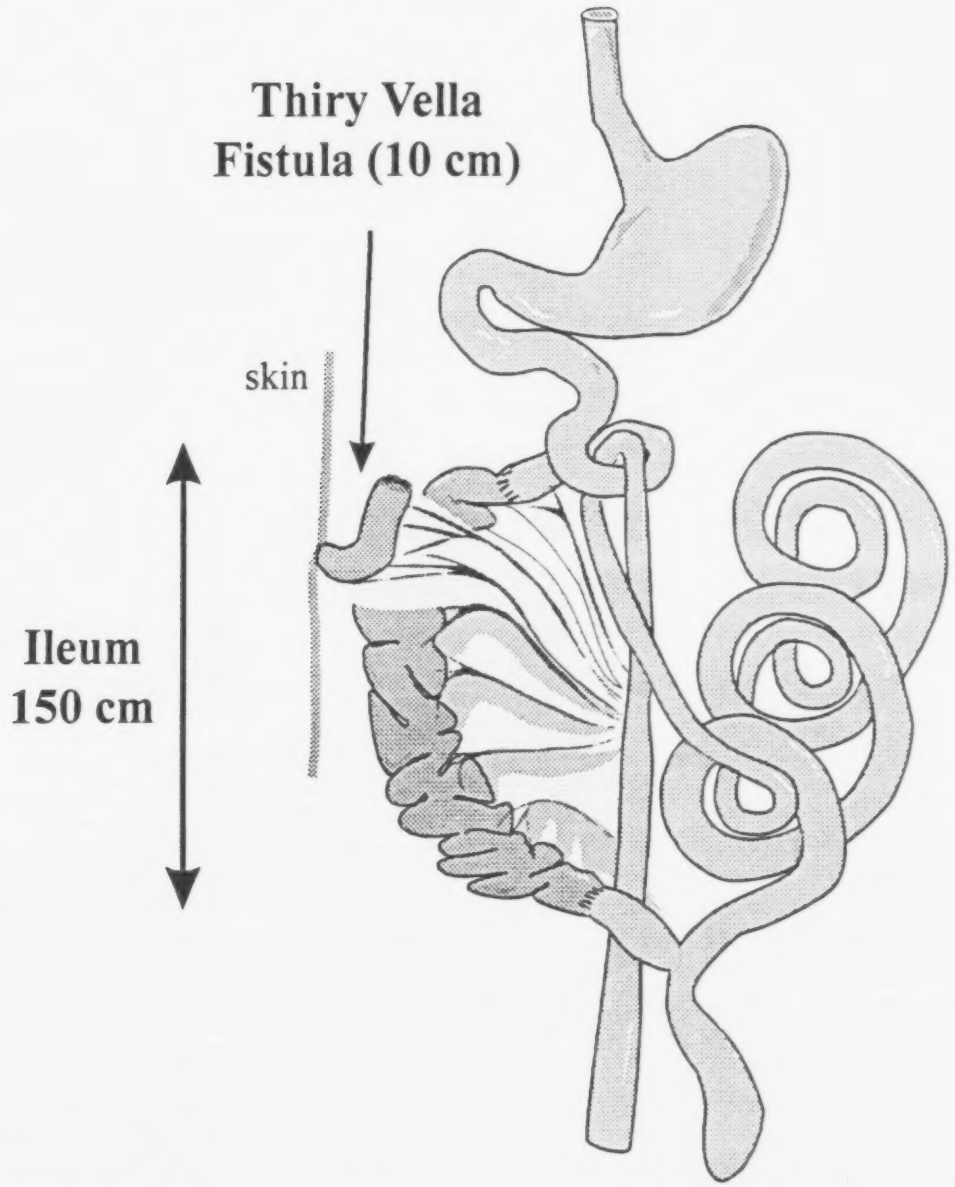


Figure 2.9 **Segmental SIT in the Pig**
Weight Gain

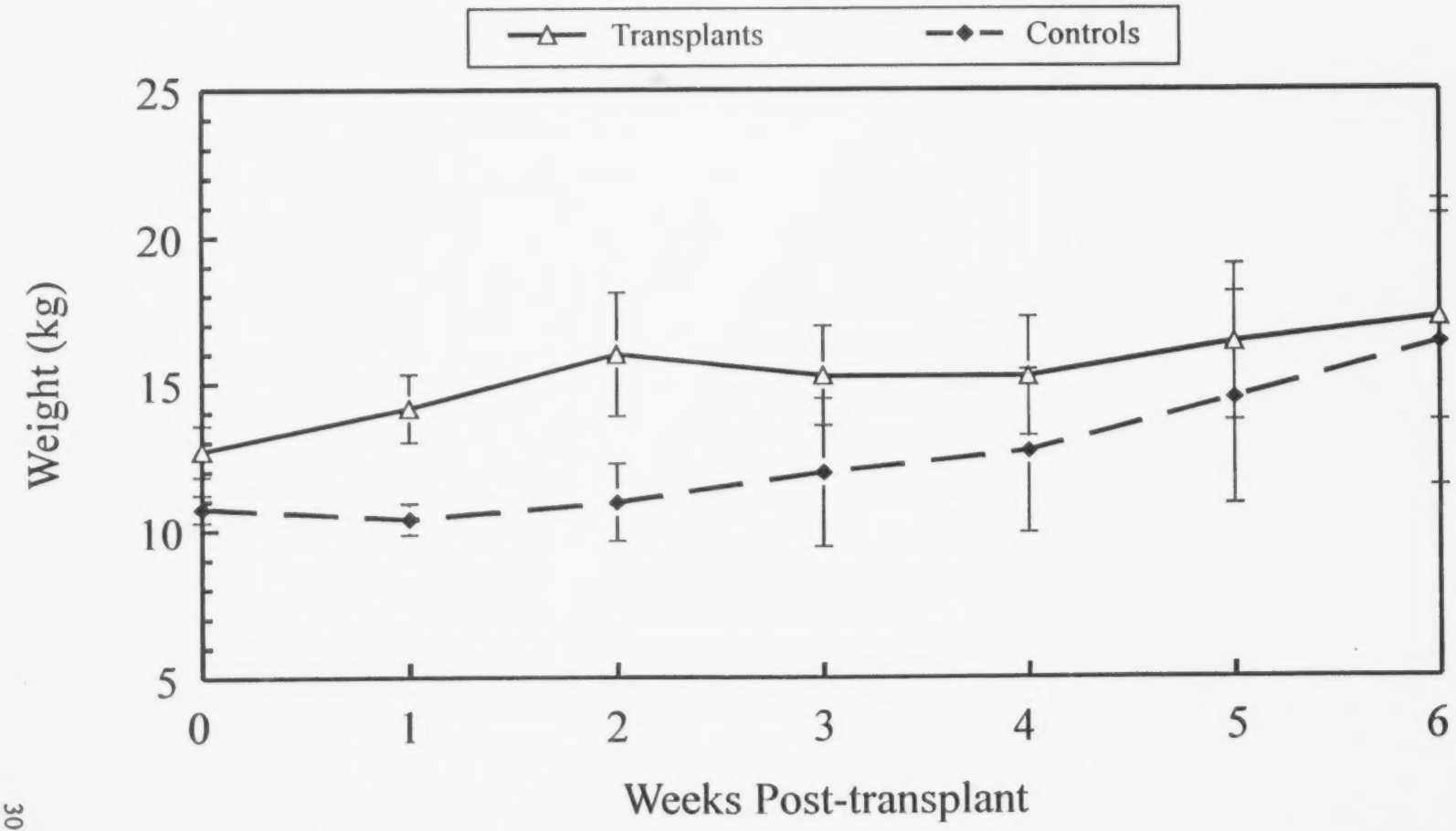
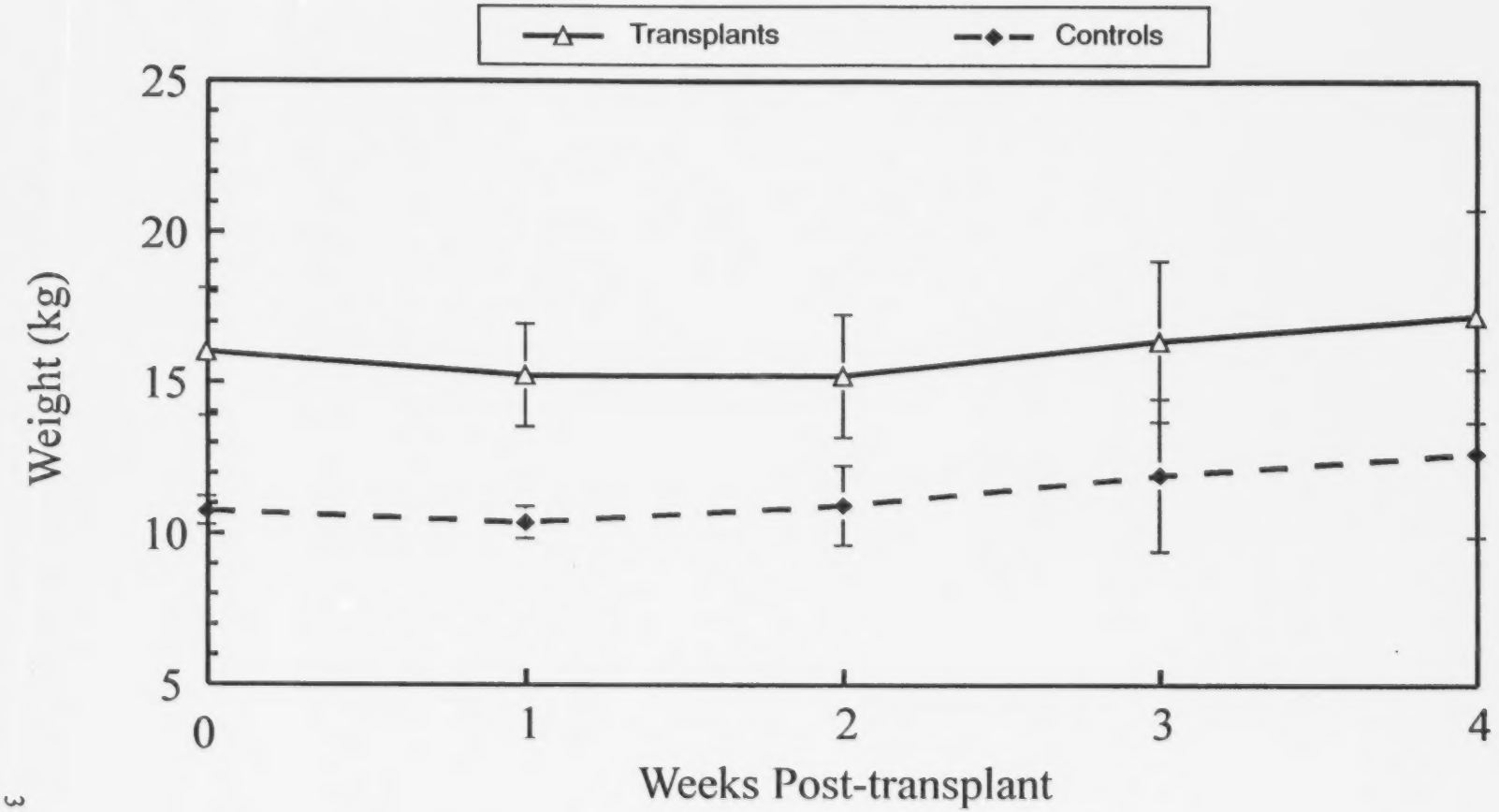


Figure 2.10 **Segmental SIT in the Pig**
Weight Gain



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SLA TYPING

Introduction

The Major Histocompatibility Complex

The Major Histocompatibility Complex (MHC) is a large gene complex that controls traits which influence the entire immune response.[1] The genes of the MHC encode cell surface glycoproteins which in humans are called Human Leukocyte Antigens (HLA). The MHC in humans consists of three different gene families. Class I and II genes code for cell-surface proteins, whereas Class III genes code for complement components. The Class I gene loci consists of HLA-A,B,C, the Class II loci include HLA-DP, DQ,DR. Class I molecules can be detected on the cell surface of almost all nucleated cells. Class II molecules can only be found on cells of the immune system. Recently Class II DR molecules have been detected on intestinal epithelial cells. [2,3] Class II antigens consist of two noncovalently associated transmembranous glycoproteins of approximately 33,000 (alpha chain) and 27,000 (beta chain) daltons. Most of the antigenicity of class II antigens is determined by the beta chain. Four genes coding for different alpha chains as well as seven genes coding for different beta chains have been identified. The alpha chain of the DR molecule is constant whereas the beta chain is variable and therefore determines the specificity of the molecule. The HLA-DR has one alpha chain and four beta chains.[3]

The immunocompetent cells of a grafted organ are susceptible to interaction with the recipient's T lymphocytes that course through the transplanted organ. In order for these lymphocytes to become sensitized an antigen presenting cell (APC) such as a macrophage is necessary. The macrophage traps, processes and presents antigens to the T cell receptor on the lymphocyte. In order for this to occur the macrophage and lymphocyte must share the same class II molecules on their cell surface. The class II of the antigen presenting cell also provides a signal which is necessary for the activation of T lymphocytes as well as for IL-1 production by the macrophage. The release of IL-1

results in the appearance of IL-2 receptors on the T cell. At the same time IL-2 is secreted by CD4+ T helper cells which interact with the IL-2 receptor allowing the T cell to proliferate and mature.[1]

Histocompatibility Testing

In transplantation the benefit of HLA matching has been shown primarily in human renal transplantation .[4,5,6] HLA- identical living related transplants continue to experience the best short and long term allograft survivals.[7] One - haplotype-matched living related transplants experienced the second best allograft survival.[8] The benefits of HLA matching in cadaveric kidney transplantation has remained controversial with some centres insisting on the significance of HLA matching for long term survival of these grafts, while others fail to find a role for it.[7]

Histocompatibility testing is possible via two methods. Serologic leukocyte testing involves using the patient's leukocytes and a bank of standard antisera to characterize the strong HLA antigens in both the donor and recipient. It is presently possible to type patients at the HLA-A, B, and DR loci. [4,5] Mixed lymphocyte cultures (MLC) detect differences at the class II locus by mixing recipient lymphocytes with those of the donor. If antigenic differences exist, the lymphocytes will react by proliferating with transformation into blast cells, DNA synthesis, and mitosis.

The Dog Leukocyte Antigen System (DLA)

In 1971 Westbroek et al showed evidence for the existence of a major histocompatibility locus in the dog.[9] In analogy to the HLA system in man this locus was called DLA. Meijssen et al in 1993 reported on their results of long-term survival of DLA matched segmental small bowel allografts in dogs.[10] They performed two-stage orthotopic segmental small intestinal transplants in DLA mismatched and DLA matched dogs. DLA matching was accomplished by serologic methods (class I) and mixed lymphocyte cultures (class II).[11,12] Postoperative immunosuppression was with

cyclosporine 15mg/kg/day im , followed by 30 mg/kg/day orally. Recipients of DLA mismatched grafts survived 113.2 days which was significantly shorter than dogs that received DLA-matched grafts which survived 211.5 days.[10]

The Swine Leukocyte Antigen System (SLA)

The pig is the ideal large animal for transplantation research due to its similarities to humans anatomically, physiologically,[13] and immunologically.[14] In order to study the benefits of living related transplantation in the pig a thorough understanding of the porcine MHC locus is necessary.

The Major Histocompatibility Complex in swine was first described by Vaiman in 1970, and was given the name Swine Leukocyte Antigen (SLA) system.[15] The genes corresponding to the SLA complex are located on chromosome 7 [16] and encode both class I and class II cell surface products.[17] Both class I and class II genes are sufficiently homologous to their human counterpart so as to be identified in southern blots using human cDNA probes.[18] Due to this degree of homology, the same nomenclature that defines the Human Leukocyte Antigen system (HLA) genes was adopted to define the corresponding swine genes. [19] The SLA-A and B loci encode the class I antigens,[16] and the class II antigens are coded by DQA, DQB, DRA, DRB. [17] Similar to its counterpart in man (HLA), SLA controls aspects of graft rejection [19] as well as other aspects of transplantation immunity including mixed lymphocyte reactions, cell mediated cytotoxicity, and antibody dependent serotoxicity.[20]

In order to study the SLA complex in more detail, Sachs and colleagues in 1976 established a herd of miniature swine that were homozygous at the MHC.[21] This group described how three strains of miniature swine that were each homozygous for a different allele of the major histocompatibility locus were developed by a selective breeding scheme based on tissue typing of the offspring of each generation. They accomplished this by reciprocally immunizing via skin grafts and lymphocyte injections the parents

prior to breeding. This resulted in the production of lymphocytotoxic antisera. These antisera were then used to assess the MHC genotype of the offspring based on selective absorption of cytotoxicity. Lymphocyte cultures were then used to verify these findings. Offspring which inherited this same genotype were then bred sequentially. A miniature swine breeding program has been maintained at the National Institute of Health since that time. In addition to the three independent MHC alleles that were derived from the founding parents, four additional intra-MHC recombinants were recognized and subsequently bred to homozygosity.[22] It is important to note that these animals are only "inbred" with respect to their MHC and should not be considered syngeneic. [21]

This miniature swine model has been used extensively to study renal allograft survival in MHC matched and mismatched animals.[23,24,25] The finding by Pescovitz in 1984 that renal vascular endothelium expressed class II antigens, as was found in humans, suggested that swine were an excellent model to study the effect of class II antigen matching on renal allograft survival. [26]

The effect of MHC matching on renal allograft survival was studied originally in the miniature swine using the entire MHC (class I and class II).[24] SLA mismatched grafts were rejected rapidly (12± 3.7 days). In addition some matched grafts were rejected (30± 15 days), however, other SLA matched grafts were accepted indefinitely. They concluded from these results that the MHC was of major importance for the survival of renal allografts, but the results also indicated that a non-SLA locus also determined antigens which could lead to kidney rejection. With the development of intra-MHC recombinants it became possible to study the effect of matching for class I only, class II only, or both class I and class II on the survival of renal allografts. [22] It was found that kidneys matched at the class I locus, and mismatched at class II for 1 or both haplotypes uniformly rejected the graft. Animals that received a kidney allograft that was class II matched, and class I completely mismatched also rejected the allograft. Kidneys that were MHC class II matched, but were single haplotype class I disparate, became tolerant to the

graft without exogenous immunosuppression in 1/3 of the cases. This group went further to show that with class II matched, and class I completely mismatched renal allografts, the addition of cyclosporine resulted in the induction of tolerance to these renal allografts. Their explanation for this was as follows: They believed that the tolerance that developed spontaneously in the 1/3 of the single haplotype class I mismatched renal allografts developed due to a limitation of donor class I specific helper cell activity at the time of the first exposure to the antigen. The cyclosporine acted by pharmacologically augmenting this endogenous helper cell deficiency.[22]

The development of this miniature swine herd that are selectively inbred at the MHC have made it possible for detailed analysis of the porcine class II gene at the DNA level to be performed. Restriction Fragment Length Polymorphism (RFLP) analysis of miniature swine using human MHC class II probes has been conducted. The miniature swine class II region was found to be very similar in organization to that of man, containing genes homologous to the human DR, DQ, DO, and DP genes. [18,19,27,28,]

A new method for rapid typing of alleles of the swine MHC DQB and DRB has recently been developed. [29] This technique utilizes SLA DQB and SLA DRB specific primers that were designed to analyze expressed genes using the reverse transcription - polymerase chain reaction method (RT-PCR). DNA products from genomic PCR amplifications were subjected to restriction enzyme digestion (PCR-RFLP), and subsequent electrophoresis. Using this method, six SLA DQB PCR-RFLP patterns have been observed using Hae III and Rsa I, and four DRB PCR-RFLP pattern types have been observed using Msp and Rsa I.[30]

We report on our experience utilizing SLA-DR typing by PCR-RFLP in an outbred pig model of Small Intestinal Transplantation.

Methods

SLA Typing

Outbred littermate pigs weighing 8-10 kg were bled of 10 mLs of blood from the vena cava, and the blood placed into heparinized blood tubes. The blood was spun at 3000 rpm for 10 minutes, and then using a Pasteur pipette, the white cell layer as well as 1/3 of the packed cell layer was carefully removed and placed into a 15 mL corning tube. RPMI was added to bring the suspension up to 7 mL and was then mixed. Ficoll-Paque (7mL) was placed into a corning tube, and the cell suspension was carefully layered on the Ficoll-Paque. The suspension was spun at 1500 rpm for 30 minutes with no brake. The mononuclear cell layer at the interface between the media and the Ficoll-paque was removed and placed into a second corning tube. RPMI (7 mL) was added to the cells and the suspension was spun at 1500 rpm for 5 minutes. The cells were placed in cryovials, and then packed on dry ice for shipment to Guelph, Ontario. The specimens were received in Guelph the following day and underwent Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR- RFLP) analysis utilizing three primers for DRB and two restriction endonucleases (Msp1, Rsa1) according to the method of Shia, Lewin, and Schook.[29,30] The procedure required 1 1/2 - 2 weeks for completion, and the results were reported as a combination of Msp 1 and Rsa 1 digestion of the DNA.

Selection of Donor and Recipient

Upon receiving the information regarding the SLA typing , transplant groups (donors/recipients) were selected as follows: SLA matched (M) (n=5), SLA haploidentical (H) (n=3), and SLA mismatched (MM) (n=8).

Transplant Procedure

Animals underwent either a single stage(n=3) or two- stage (n=13) segmental ileal intestinal transplant. Portocaval drainage was used. In the single stage procedure the allograft was placed in the orthotopic position following revascularization. In the two-stage procedure, the allograft was initially placed heterotopically. Two weeks later , the native intestine was removed ,and the allograft placed orthotopically.

MLC Verification

The donor and recipient were bled of 10mL of blood at the time of transplantation, and the blood placed in heparinized tubes. The blood was spun at 3000 rpm for 10 minutes, and then using a Pasteur pipette, the white cell layer as well as 1/3 of the packed cell layer was carefully removed and placed into a 15 mL corning tube. RPMI was added to bring the suspension up to 7 mL and was then mixed. Ficoll-Paque (7mL) was placed into a corning tube, and the cell suspension was carefully layered on the Ficoll-paque. The suspension was spun at 1500 rpm for 30 minutes with no brake. The mononuclear cell layer at the interface between the media and the Ficoll-paque was removed and placed into a second corning tube. RPMI (7 mL) was added to the cells and the suspension was spun at 1500 rpm for 5 minutes. The cells were resuspended in 1 mL of RPMI containing 10% matching serum. Two drops of trypan blue were added to two drops of cell suspension, and a cell count performed with a hemocytometer. Negative and positive controls were set up as well. Stimulator lymphocytes were exposed to 30 Gy (3000 rads) for 4 minutes. To a 96 well - round bottomed plate, 0.1 mLs of stimulator cells, and 0.1 mLs of appropriate responder cells were added. The plate was placed in 37 C, 5% C02 for 7 days. The cells were pulsed after maximal stimulation with 3H-Thymidine, and were then reincubated at 37°C for 18 hours. The cells were harvested using a cell harvester. The filter paper was allowed to dry overnight, and then the disks

were placed into scintillation vials. The Beta radiation emitted was counted using a Beta-scint counter. Results were expressed as median count of the three samples counted.

Immunosuppression

All animals received methylprednisolone 1 mg/kg iv daily for 3 days post-op. Cyclosporine was given initially iv, and was subsequently changed on day 5 to oral cyclosporine. The initial starting dosages were 7 mg/kg/day iv, 20 mg/kg/day orally following stage I, and 30 mg/kg/day orally following stage II. Cyclosporine was dosed twice daily, and all doses were adjusted to maintain a target twelve hour whole blood trough level of 250-350 ng/mL.

Postoperative Care

All animals were housed initially in metabolic cages, and were allowed nothing by mouth for the first 48 hours. During this time they received 500 mL of iv D5 Saline + 20 mEq KCl/L twice a day. In addition they received Cefazolin 500mg iv, and Ranitidine 25 mg iv , each twice a day. Buprenorphine 0.4 mg iv twice daily was given for analgesia. These medications were given for the first 48 hours post-op. On post-op day 3 , the animals were allowed water ad lib, and were then progressed to porcine mix the following day, and then started on pig chow on post-op day 5. They were then continued on pig chow and water ad lib until the end of the study period. In addition , all animals were supplemented with enteral feeding (Vivonex).

Monitoring

a) <u>Cyclosporine Levels</u>

Whole blood cyclosporine levels were determined using the Abbott TDX monoclonal Radioimmunoassay. Levels were determined twice weekly for the first week, and subsequently weekly.

b) Weight Gain

Animals were weighed weekly. Weight gain was expressed as a percentage of the starting weight for the control animals. In the transplant group weight gain was expressed as a percentage of the pre-op stage II weight (i.e. reflecting the time that the allograft was in the orthotopic position)

c) General Well-Being

All animals were monitored daily for feed intake, excretions, and general well being. Animals were observed daily for signs of GVHD (dermatitis, diarrhea, lymphadenopathy), or rejection (weight loss, diarrhea, decreased appetite, lethargy)

Determination of Study Endpoint

There were three possible ways that the study could be ended:

1. Animals that survived the 6 week study period were euthanized at the time of the final laparotomy.

2. At the time of the second stage procedure, if the allograft was determined to be nonviable, the animal was euthanized at that time.

3. If during any other time in the study the animal looked unwell including either weight loss > 10% of the starting weight, continued loss of appetite, persistent diarrhea, persistent vomiting, or persistent lethargy, then the animal was euthanized.

Results

Thirty-two outbred pigs (4 litters) underwent Swine Leukocyte Antigen (SLA) typing of their DR locus. Results were obtained for 27 animals based on the first blood sample, however, 5 animals required a repeat sample due to poor DNA amplification. One new pattern (donor for T11) emerged that had not been observed previously, but was considered different (i.e. mismatched) from all other patterns.

A total of 16 Small Intestinal transplants (table 3.1) were performed. Transplant animals were divided into matched (n=5), haploidentical (n=3), and mismatched (n=8) on the basis of SLA typing. T1-T3 underwent single stage Small Intestinal Transplants (SIT), that were unsuccessful due to technical complications (n=2), and infection (n=1). The two stage procedure was performed on thirteen animals. In the matched group (n=4), there was one technical death (T13), one animal underwent rejection of its allograft (T12). Two animals survived until the end of the study period (T4, T14). In the mismatched group (n=6), there was one anesthetic death (T8), one technical death (T16), two animals rejected their allografts (T9, T11) and two others survived (T10, T15)until the end of the study period. In the haploidentical group (n=3), animals survived a mean of 15 days, with two animals (T5, T6) developing lethal Graft versus Host Disease (GVHD) , and one animal (T7) succumbing to severe rejection.

Mixed Lymphocyte Cultures (MLC) were performed in 7 of 16 transplants (T1, T3, T4, T5, T6, T7, T8). The MLCs were performed in three runs. In Run #1 (table 3.2), negative controls ranged from 5.3-27 cpm, positive controls ranged from 409- 1526 cpm. The test animal (T1) had a positive MLC (550 cpm). In run #2 (table 3.3) negative controls ranged from 13-32 cpm, and positive controls ranged from 723- 1400 cpm. The results of the test animals were as follows: T3 (112 cpm) and T4 (118 cpm) had negative reactions, while T5 (252 cpm) was considered to have a reaction almost half-way between positive and negative. In run #3 (table 3.4) negative controls ranged from 728-850 cpm, and

positive controls ranged from 1865- 2680 cpm. The test animals, T6 (1747), T7 (2408), and T8 (2287) all had positive reactions.

Discussion

The MHC locus of the pig has been studied extensively in miniature swine. The class II region has been found to be very similar in organization to that of man.[18] There are certain disadvantages to using miniature swine in research of small bowel transplantation. These include expense, small litter sizes, and slow weight gain. In addition because they are inbred at the MHC, the possibilities for variability at this level are decreased unlike the human situation. In order to overcome these problems, outbred swine were chosen as the study animal. Due to recent technology that has been developed , it is now possible to type outbred swine at the DR locus.[29,30]

The process of obtaining, preparing, and shipping of the blood sample was satisfactory. A 10 mL blood sample was easily obtained from the inferior vena cava without too much stress on the animal. It had been decided to have the SLA typing performed in Guelph, Ontario, as this was one of only a few centres presently performing this procedure. It was therefore necessary to devise a method to prepare and freeze the lymphocytes for shipment in a way so as to minimize damage to the DNA. This was accomplished by the addition of RPMI and Ficoll-Paque to the samples which allowed for better separation of the WBC as well as preservation of the DNA . The samples were sent in litters (8-9 pigs/litter), and the turnaround time to receive the results was two weeks. It was necessary to take this time period into consideration as pigs gain approximately 2 kg per week, and the target starting weight for our animals was 10-15 kg.

SLA typing results could be obtained on all animals tested, however there were a few animals that required repeat testing, due to insufficient amplification of the DNA. This occurred early on in the experiment, and was felt to be due to the early attempts at shipping lymphocytes prior to adding RPMI and Ficoll-Paque to the sample. After this modification was carried out, all samples amplified.

MLC was performed on 44% of the animals. It confirmed the SLA typing in a total of 71% of the cases. It did , however, confirm the SLA typing in 100% of the matched animals tested reliably placing their reaction in the range of the negative control reactions, supporting the lack of difference, and hence the lack of immunological reactivity between these animals. MLCs also confirmed the SLA typing in 100% of the mismatched animals, reliably placing their reaction in the range of the positive control groups supporting the difference between the groups immunologically. In the haploidentical group, however, MLC confirmation occurred in only 33% of the animals. The MLCs that did not confirm the SLA typing in the haploidentical group were performed in a run (table 3.4) that was of questionable accuracy, due to high negative controls. The MLC from the third haploidentical transplant was performed in a different run, and it had a reaction approximately half-way between the negative and positive reactions, thus confirming the SLA typing in this transplant.

SLA typing of the DR locus only provides information regarding Class II genes. The information obtained from the MLC also only applies to the Class II genes. It is possible that even in the animals that are matched at the Class II locus, there could be a mismatch at the Class I locus . However, the fact that these animals are littermates makes it unlikely that animals matched at Class II would be completely mismatched at Class I.[31] Furthermore, studies have shown that the addition of cyclosporine induces tolerance to Class II matched, Class I mismatched porcine renal allografts.[22]

Table 3.1- SLA Typing Results

Animal ID	Donor MSP	Donor RSa	Donor combo	Recipient MSP	Recipient RSa	Recipient combo	MLR	Outcome
T3	c/c	b/b	6/6	c/c	b/b	6/6	conf	infection
T4	c/c	b/b	6/6	c/c	b/b	6/6	conf	survival
T12	a/a	d/d	5/5	a/a	d/d	5/5	N/A	rejection
T13	a/d	d/d	5/5	a/a	d/d	5/5	N/A	technical
T14	a/c	b/b	1/6	a/c	b/b	1/6	N/A	survival
T5	a/a	b/d	1/5	a/a	b/b	1/1	conf	GVHD
T6	a/a	b/b	1/1	a/a	b/d	1/5	not conf	GVHD
T7	a/a	b/b	1/1	a/a	b/d	1/5	not conf	rejection
T1	c/c	b/b	6/6	a/d	d/d	5/5	conf	technical
T2	a/a	d/d	5/5	a/a	b/b	1/1	N/A	technical
T8	a/a	d/d	5/5	c/a	b/b	1/6	conf	anesth death
T9	c/c	b/b	6/6	a/a	d/d	5/5	N/A	rejection
T10	a/a	b/b	1/1	c/c	b/b	6/6	N/A	survival
T11	new pattern	new pattern	N/A	not available	not available	1/5	N/A	rejection
T15	c/c	b/b	6/6	a/a	e/e	5/5	N/A	survival
T16	a/c	b/b	1/6	not available	not available	1/1	N/A	technical

Matched
T3, T4, T12, T13
T14

Haploidentical
T5, T6,T7

Mismatched
T1, T2, T8, T9, T10
T11, T15, T16

MsP, Rsa - Restriction endonucleases
combo- combination of results of MsP and Rsa digestion

MLC Results

Table 3.2 - MLC Run #1

Negative Controls	Positive Controls	Test Animals
C12 vs. C12 5.3 cpm	C12 vs. D06 1526 cpm	C12 vs. C11 (T1) 550 cpm
C11 vs. C11 27 cpm	D06 vs. C11 1650 cpm	
	C11 vs. D06 409 cpm	

Table 3.3 - MLC Run #2

Negative Controls	Positive Controls	Test Animals
B vs. B 27 cpm	D8 vs. A 922 cpm	B vs. A (T5) 252 cpm
D8 vs. D8 13 cpm	B vs. D4 723 cpm	D8 vs. D4 (T3) 112 cpm
D6 vs. D6 32 cpm	D6 vs. B 1400 cpm	D6 vs. D5 (T4) 118 cpm

Table 3.4 - MLC Run #3

Negative Controls	Positive Controls	Test Animals
C vs. C 850 cpm	C vs. D1 1865 cpm	C vs. G (T6) 1747 cpm
D vs. D 728 cpm	D vs. D1 2680 cpm	D vs. H (T7) 2408 cpm
E vs. E 841 cpm	E vs. D1 2251 cpm	E vs. F (T8) 2287 cpm

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IMMUNOMODULATION OF INTESTINAL TRANSPLANTS USING RELATED DONORS

Introduction

EXPERIMENTAL IMMUNOMODULATION OF INTESTINAL TRANSPLANTS

Pre-Cyclosporine

Since Lillehei in 1959 developed the procedure for allografting small bowel, researchers have been working on methods to prevent rejection in this procedure. It is felt that one of the reasons that the small bowel is so difficult to successfully transplant is due to the fact that it contains a large amount of lymphatic tissue, thus bringing about a large immunologic reaction. It was the general experience in the early 1960's that intestinal allografts in dogs that were untreated would generally survive six to nine days.[1] Taylor in 1965 transplanted jejunal homografts into the neck of 10 recipient dogs, 5 of these dogs were treated with azathioprine. The dogs that were treated with azathioprine showed a relatively long survival ranging from 21 to 42 days. [2] Preston in 1966 was able to prolong the survival of 13 intestinal allografts to 59 days using azathioprine and prednisone. [3] Hardy in 1969, studied the survival of cervical jejunal autografts and allografts. In addition to studying the morphological aspects of the graft, functional assessment of the graft was ascertained as well. Immunomodulation was accomplished using anti-lymphocyte serum, prednisone, and azathioprine. This experiment showed that this combination of immunotherapy did result in successful prolongation of both morphological and functional jejunal allograft survival. The longest graft survived 54 days (function) and 59 days (gross appearance).[4] By 1972, Lillehei, with the use of azathioprine and prednisone was able to keep his dogs with intestinal allografts alive for more than 3 weeks which allowed him to observe for the first time the classic signs of rejection in the entire intestinal allograft. These consisted of: mucosal bleeding, sloughing of the mucous membranes, disappearance of the villi, multiple ulcerations, perforation, thickening of the bowel wall, and perivascular cellular infiltration in the submucosa.[5] In 1976, Cohen, working with the canine small bowel allograft model was

able to show that when allografts were treated with 50 rads of radiation prolonged survival of the recipient to a mean of 28 days was possible. [6]

Cyclosporine

In 1978, at the 7th International Congress of the Transplantation Society in Rome, Sir Roy Calne of Cambridge reported on a handful of clinical cases of cadaver kidney recipients who had been started on cyclosporine since the end of June 1978.[7] This was probably one of the most important announcements in transplantation history as it would be the introduction of cyclosporine that would allow liver, kidney, heart and lung transplants to become a success.

Mechanism of Action

Cyclosporine is a neutral lipophillic cyclic endecapeptide possessing a molecular weight of 1203 that is extracted from the fungus tolypocladium inflatum. It is felt that the amino acids in positions 1, 2, 3, & 11 form the active immunosuppressive site. [8]

Cyclosporine exerts its effects on responding T cells by preventing their response to interleukin-2 (IL-2), however it does not inhibit the response of activated T cells to IL-2. It has been postulated that as T cells become sensitive to IL-2 through signals from the HLA-DR of stimulator cells, then cyclosporine blocks receptors for DR molecules on the responding T cells and thus inhibits the expression of IL-2 receptors on the same cells.[9]

Oral cyclosporine is predominantly absorbed in the small intestine. The rate of absorption is dependent on emulsification of the drug and its vehicles by bile acids. Once in the vascular compartment, the major drug fraction becomes cell-associated, and the largest serum fraction binds to lipoproteins, with a very small free fraction. After the drug is taken up by the liver it is metabolized by the cytochrome p-450 system producing metabolites that are primarily excreted in the bile (94%) as well as in the urine (6%).[10]

Cyclosporine Monitoring

It was recognized that clinical response to cyclosporine did not correlate well with the administered dose.[11] It was for this reason that cyclosporine concentration in the blood became essential for optimization of therapy. In 1987 it was recommended by the Task Force on Cyclosporine Monitoring that for routine monitoring of cyclosporine concentrations, whole blood would be the preferred matrix.[11] In addition it was recommended that a specific assay that measures the parent cyclosporine and not its metabolites be used.[11] Cyclosporine concentration in the blood can be measured by either high performance liquid chromatography (HPLC) or by radioimmunoassay (RIA). HPLC was the first specific method developed for the analysis of cyclosporine.[12] It has proven to be rapid, accurate, and precise, however it was faulted due to its technical difficulty.[12,13] The original radioimmunoassay kit produced by Sandoz made use of a polyclonal antibody serum which cross-reacted with the metabolites of cyclosporine.[14]

Due to the demand for an RIA with less cross-reactivity to metabolites, Sandoz introduced a replacement for its original RIA called the Sandimmun-Kit which was based on two monoclonal anti-cyclosporine antibodies and which exhibited minimal cross-reactivity with cyclosporine metabolites.[14,15] In response to increased demands for cyclosporine analysis it was necessary to develop a commercially available reliable automated assay for selectively measuring cyclosporine in whole blood. The Abbott TDx was introduced for this purpose.[15] It is an automated assay for cyclosporine involving fluorescence polarization technology. It originally used a polyclonal antibody which showed considerable cross-reactivity with cyclosporine metabolites.[13] A monoclonal antibody with a high specificity for cyclosporine was subsequently used for this assay, and this now provides for a rapid, accurate, precise, and less labor intensive monitoring method for cyclosporine.[15]

The Impact of Cyclosporine on Experimental Small Intestinal Transplantation

1. Cyclosporine use in Canine SIT

Up to and including the late 1970's despite many researcher's efforts, long term survival of small intestinal allografts had not been possible. It was not until the early 1980's, when cyclosporine was introduced that researchers began to make progress in this field. Cyclosporine had been successful in prolonging survival of kidney, heart and liver transplants,[16,17] and it had also been shown to be effective in preventing and treating Graft Versus Host Disease (GVHD) in bone marrow transplants.[18] Resnick in 1981 undertook an experiment in dogs in which he studied the efficacy of cyclosporine in prolonging survival of these animals after small bowel transplantation.[19] The 10 control animals survived a mean of 12.5 days whereas the 11 treated animals had an increased survival with a mean of 90.6 days. From this experiment Resnick was able to show that the drug was effective in prolonging graft and animal survival following small intestinal transplantation. In addition, they noted useful bowel function in 9 out of the 11 treated dogs manifested by weight gain, near normal xylose tolerance test and normal appearing formed stool. Craddock, in 1983 chose to study the effects of cyclosporine on survival in dogs after small bowel transplantation, and in addition compared different routes of administration.[20] He used the same model as Resnick and divided his animals into 3 groups. Like Resnick, his first group received no immunosuppression, and a second group received cyclosporine 25 mg/kg by intramuscular route, but in addition he also had a third group to which he administered cyclosporine 25 mg/kg per day by oral route starting from the first day post-op. The transplants in the second group which had received intramuscular cyclosporine showed a mean survival of 103.8 days in 11 of the surviving dogs and in addition 3 animals survived 203, 221 and 442 days. The third group in which the cyclosporine was given orally showed a mean survival of 30.4 days. From this study it was shown that the combination of early intramuscular cyclosporine followed by oral cyclosporine is more effective than initially starting with cyclosporine

by mouth, probably due to the fact that absorption is unpredictable in the early post-op period following a small intestinal transplant.

2 . Cyclosporine use in Porcine SIT

Over the past decade the pig has become a very popular animal for experimental small bowel transplantation. In fact, successful intestinal transplantation in pigs appeared to be a prerequisite in some centres prior to initiation of clinical small bowel transplantation programs.[21,22] Pigs are an excellent model for small bowel transplantation. Their gastrointestinal anatomy and physiology is similar to human's.[23] The patterns of intestinal rejection and GVHD in swine are comparable to patterns seen in human.[24] The first group to study the effect of cyclosporine on the survival of pig small intestinal allografts was Ricour et. al. in 1983. [21] In this landmark article, this group studied the routes of administration of cyclosporine and their effects on the survival of the small bowel. They studied two different models of small bowel transplants, a double enterostomy model where the allograft was not in continuity with the native bowel, and a second group the continuity model, where the allograft was immediately hooked up to the remaining native bowel. They found that when no cyclosporine was given, or when given every other day, acute rejection occurred anywhere from 5 to 20 days post-op. When they studied the effect of oral cyclosporine on the rejection process they found that when the cyclosporine was being absorbed through the allograft in the continuity model, 6 out of 7 cases showed an acute or delayed rejection reaction, in comparison to the double enterostomy model where 4 out of 13 cases presented with a subacute rejection. The cyclosporine levels of 150 - 300 ng/mL in the continuity model vs. the 250 - 2000 ng/mL in the double enterostomy model explains why there was a much higher rate of rejection in the former. They attributed this decreased absorption in the allograft to severing of the lymphatics during the transplantation process. It was felt that only by 2 -8 weeks later had the lymphatics regenerated to the point that absorption was back to normal. Their final group of pigs that received IV cyclosporine for 5 - 10 days and then switched to oral

cyclosporine, showed long term survival with no graft rejection observed on days 100, 150 and 200 in the three pigs respectively. They concluded from this study that the success of intestinal transplantation depends on intravenous administration of cyclosporine until normalization of intestinal transport occurs. Grant's group in 1988 reported on successful intestinal transplantation in pigs treated with cyclosporine.[22] This group used continuous IV cyclosporine followed by oral cyclosporine in all their animals receiving immunosuppression. They performed orthotopic small bowel transplantation and divided their pigs into 4 groups. Group 1 received no immunosuppression, Group 2 received cyclosporine 8 mg/kg/day IV for 3 weeks and then started on oral cyclosporine 25 mg/kg/day, group 3 received cyclosporine 20 mg/kg/day for 3 weeks and then converted to oral cyclosporine 25 mg/kg/day. Group 4 received cyclosporine 15 mg/kg/day for 7 - 10 days and then changed to oral cyclosporine 30 mg/kg/day and tapered to 15 mg/kg/day over 3 - 4 months. Their results were as follows: Group 1 animals survived for a mean of 5.3 days; Group 2 animals survived for a mean of 11.3 days; Group 3 survived a mean of 28 days. The higher dose of cyclosporine in Group 3 prevented rejection but most pigs died of bacterial infection. Group 4 animals showed a mean survival of 121 days. This regimen of immunosuppression in group 4 was felt to prevent rejection reliably. This final group had histologically normal grafts, normal nutritional indexes and normal growth rates. Unfortunately, this final group had extremely high levels of cyclosporine, up to 3000 ng/mL. Although Grant's study and others since[25] have shown that long term survival of small bowel allografts is possible in the porcine model using cyclosporine, the cyclosporine levels that have been attained have been very high and could not be tolerated in humans because of toxic side effects.

In order to be able to apply this model to clinical situations it was necessary to provide some form of immunomodulation so that successful long term small bowel transplantation could be accomplished using low enough doses of cyclosporine to be tolerated in human beings. In an attempt to do just that, Kimura studied the

immunological advantages of short segments of bowel together with low doses of cyclosporine.[26] Their cyclosporine dosage was 10 mg/kg intramuscularly for 100 days, then on alternate days. The segmental graft that they used consisted of 3 to 4 metres of jejunum with a vascular pedicle consisting of 1 jejunal artery and 1 vein. The segmental transplants that were treated with low dose cyclosporine survived an average of 80.9 days compared to 8.8 days in those that did not receive immunosuppression. The animals that survived gained weight and thrived. This study shows that segmental small bowel transplantation utilizing low dose cyclosporine is possible. However, if one were to use a segment of bowel it may be more advantageous to use a segment of ileum, as without ileum there would be some concern regarding vitamin B12 absorption as well as bile salt absorption. This study did not examine the absorption of orally administered cyclosporine. It is unclear whether a segmental jejunal allograft would be capable of absorbing sufficient amounts of orally administered cyclosporine to attain appropriate therapeutic drug levels.

In a further attempt to utilize low dose cyclosporine in porcine small bowel transplantation, Alsnia et. al. combined cyclosporine, azathioprine and solumedrol in an attempt to attain long term graft survival.[27] They used cyclosporine 5 mg/kg/day intravenously for the first 3 weeks and then used cyclosporine to keep the 24 hour trough level at 300 - 400 ng/mL. Only 6 of the 28 (21%) reached the end of the 90 day study period. The mean survival of 63 days in this study is an accomplishment considering that long term survival was previously only possible with higher dosed cyclosporine. One must examine though what is meant by "low dose cyclosporine". This study indicated that they used initially 5 mg/kg intravenously but then adjusted their cyclosporine levels after 3 weeks to attain levels of 300-400 nanograms/mL which they state was a 24 hour trough level. If one were to look at some of the pharmacokinetic studies that have been done on porcine cyclosporine metabolism[22] then one would see that a 24 hour trough level of 300-400 nanograms/mL translates to a much higher 12 hour trough level, and it is

the 12 hour trough level that is used in human transplantation with a therapeutic range of 300-400 ng/mL.

Recently with the introduction of the new potent immunosuppressant FK506, researchers have found that in rat small intestinal transplantation, this drug demonstrates superiority to cyclosporine with respect to preventing graft rejection and abolishing GVHD.[28]

Researchers studying the effects of FK506 on SIT in pigs were not as successful at demonstrating its superiority over cyclosporine. Gruessner and colleagues performed orthotopic SIT in pigs and immunosuppressed them with either FK506 (0.2 mg/kg/day) or CsA-ATG (CsA 3mg/kg/day- ATG 10mg/kg/day). They found that the FK506 group had a stronger tendency toward rejection than the CsA group. However, there was a significantly longer survival in the FK506 group which was felt to be due to an increased infection rate in the cyclosporine group.[29] It might have been more advantageous to compare FK506 to cyclosporine alone in porcine SIT, as the addition of ATG prevents a proper comparison between FK506 and CsA.

IMMUNOMODULATION OF CLINICAL INTESTINAL TRANSPLANTATION

Pre-Cyclosporine

The first complete intestinal transplant was performed by Lillehei in 1967 at the University of Minnesota.[5] The patient was a 46 year old female who had developed intestinal infarction due to mesenteric venous thrombosis. He resected the intestine from the ligament of treitz to the distal rectum. A cadaver donor was used and the bowel was harvested from the ligament of treitz to the mid transverse colon. An end to side SMV to iliac venous anastomosis was performed, as well as an end to side SMA to iliac artery anastomosis. The colour of the graft returned to normal. A jejunostomy and colostomy were performed with the proximal and distal ends (to be connected at a later date). The patient went in to shock post-operatively and died 12 hours after surgery. Autopsy

showed pulmonary congestion and pulmonary infarcts, portal vein thrombosis, early ischemia - necrosis of the liver, the transplanted intestine showed venous congestion but both anastomoses were patent.

Okamura and associates in 1968 performed a partial (1.7 m) heterotopic small intestinal transplant with total resection of the small bowel and left colon due to SMA thrombosis. [1] They performed an SMA to left iliac end to end anastomosis as well as an SMV end to end anastomosis. Proximal and distal stomas were constructed. The allograft became necrotic on the sixth post-op day. It was evident that early rejection was present in the grafted intestine.

In 1969 Oliver and associates performed an intestinal transplant on a 35 year old male who had Gardner's syndrome.[5] The recipient had the jejunum, ileum and right transverse colon resected. The allograft consisted of the entire small intestine (except the duodenum) and the right and transverse colon. The SMA was anastomosed to the recipient SMA, and the donor SMV was anastomosed to the recipient SMV. Gastrointestinal continuity was re-established and the patient was put on azathioprine, steroids and ALG post-operatively. Unfortunately the allograft became necrotic by the 23rd day and the patient died of septic shock. Autopsy revealed that the mucosa was hemorrhagic and ulcerated, the intestinal wall and mesentery were thickened and the mesenteric lymph nodes infarcted. The vascular anastomoses were patent, and mononuclear infiltration and necrosis involving all areas of the graft as well as capillary and venular thrombosis were present.

In 1969, Alkin and Hardy performed the first living related intestinal transplant. [1] They operated on a 10 year old male whose small bowel from the ligament of treitz to the ileocecal valve was gangrenous secondary to strangulation by a mesenteric band. The involved bowel was resected. The donor graft consisted of a one metre segment of ileum taken from the child's mother who was a tissue match on the Tarasaki scale. The recipient's iliac veins and lower IVC had been infarcted, and therefore the SMV of the

donor was anastomosed to the left renal vein of the recipient, and the SMA of the donor was anastomosed to the recipient's aorta. Both ends of the graft were brought out as stomas. The patient was given azathioprine, prednisone, and ALG post-operatively. On the 7th postoperative day it was noticed that the stoma appeared necrotic and the patient became septic. The graft was removed but the patient died of sepsis. The microscopic examination of the grafts showed extensive ischemic necrosis.

In 1970, another living related transplant was performed by Fortner and associates.[30] A 37 year old female underwent a massive intestinal resection for multiple intestinal polyposis. The donor allograft consisted of a 1.5 metre segment of lower jejunum and upper ileum removed from the patient's sister (after tissue typing). The SMV was anastomosed to the right iliac vein and the SMA was anastomosed to the right iliac artery. Gastrointestinal continuity was restored and the patient was started on azathioprine and prednisone post-operatively. Two months after the procedure the patient was tolerating a low fat diet. On the 75th day post-op, the patient developed GI bleeding, became acidotic with signs of pulmonary emboli. She died 79 days after transplant.

The results of small bowel transplantation up to this point had been dismal with no long term survivors. By the mid 1970's the enthusiasm for intestinal transplantation had decreased significantly.[31] There was not much progress in the field of Intestinal Transplantation until the cyclosporine era in the mid 1980's.

Cyclosporine

The next wave of clinical intestinal transplants began in 1985 in Toronto where intestinal transplantation was performed with cyclosporine.[32] The graft survived for 12 days and the patient eventually died of hypotension. In 1987 and 1988 small intestinal transplantation was performed in Chicago; Kiel, Germany; and London, Ontario; with graft survival of 10, 12, and 15 days respectively.[31] Goulet, Revillon et. al. from Paris reported the results of a small series of small intestinal transplants that had been

performed since the start of their clinical program in 1987. [21]They reported on 6 small intestinal transplants in 5 children ranging in age from 5 months to 9 years old. A segment of 110+/- 10 cm of jejunoileum was used as the allograft in all cases. Immunosuppression consisted of Cyclosporine, Methylprednisolone, ATG and Azathioprine. The graft survival results were as follows: One graft lasted 3 hours due to necrosis and was retransplanted. Three grafts succumbed to rejection at 3 weeks, 2 months, and 17 months post-transplant, and one graft suffered from visceral failure. The 5th cases, a five month old girl had survived two-and-a half years at the latest reported follow-up. At that time she remained totally fed enterally by mouth and was growing normally.[33]

In the late 1980's several combined liver/small intestinal transplants were performed. Included in this group were 2 in Chicago, the graft surviving 4 and 90 days, one in Pittsburgh where the graft survived 210 days but unfortunately the patient died of sepsis; and one in London Ontario that has survived five years to date.[31,34,35,36]

During the 1990's small intestinal transplantation has been reported by 6 centres. They include: London, Ontario;[34,35,36] Paris, France;[33,37] Kiel, Germany;[38] Pittsburgh, Pennsylvania;[39,40] Omaha, Nebraska;[41] and Manchester, United Kingdom.[42]

The largest series reported so far has been by Todo, Tzakis et. al. from Pittsburgh in 1992.[43,44] Between May 1990 and November 1992, 30 patients underwent intestinal transplantation at the Pittsburgh Transplant Institute. The patients consisted of 15 adults, and 15 children. FK506 was used in all transplants. Three types of procedures were carried out. Isolated small intestinal transplants (n=9), combined liver/ small intestinal transplants (n=17) , and abdominal multi-visceral transplants (n=4). The results were as follows: In the isolated small intestinal transplant group, 8 patients (88%) were alive 8-15 months post-op and 7 patients were maintaining their nutritional requirements without TPN. The second group, combined liver/small intestinal transplants, 13 patients (76%) were alive 5-30 months post-op, 4 patients had died. The patients that died were all

pediatric patients, 3 succumbing to sepsis and one to lymphoproliferative disease. The third group of abdominal multi-visceral transplants were all alive with functioning grafts. This same group presented their 4 year follow-up on these patients recently. The 24 month graft survival for isolated small bowel, combined liver/ small bowel, and multi-visceral transplants were 48.2%, 55.4%, and 57.7% respectively. They concluded that although FK506 allowed clinical intestinal transplantation to become a reality, the results were still unsatisfactory, and to overcome this problem a new immunosuppressive strategy would be needed.[45]

IMMUNOLOGICAL RESPONSE TO SIT

Graft Versus Host Disease

Small bowel allografts contain a considerable number of immunocompetent cells in both Peyer's patches and mesenteric lymph nodes.[46] Due to this large amount of lymphoid tissue, an intestinal allograft, more than almost any other organ with the possible exception of bone marrow and spleen, can induce Graft Versus Host Disease (GVHD).[47] It was Billingham in 1959 who first defined the prerequisites for the development of GVHD. He included: the presence of mature, immunologically competent cells in the graft, sufficient time for these cells to react before they are rejected by the host, and important histocompatibility antigens in the recipient that are lacking in the transplant.[48] Researchers as early as 1959 suspected that GVHD would be a major obstacle to overcome in small bowel transplantation.[48] In order to investigate this phenomenon further, Monchik and Russell in their landmark article used parenteral strain donor rats and F1 hybrid recipients from inbred rat strains which resulted in a unidirectional GVH reaction.[49] These animals experienced progressive weight loss and alopecia followed by the development of cutaneous erythema and scaling. The spleens of these animals showed a loss of the normal follicular pattern with depletion of lymphocytes and the appearance of histiocytes. Examination of their lymph nodes

revealed loss of the usual architecture with an increase in the plasma cells of the medulla. Donor irradiation prevented the appearance of the GVH reaction in the F1 recipients indicating that this GVH response was mediated by the lymphoid cells of the graft.

GVHD when precipitated by a small bowel allograft, follows a predictable pattern that involves primarily, the skin in the form of dermatitis, the host's lymphatic tissue (lymphocytic depletion, loss of follicular architecture), as well as the host's own intestine in the form of necrotizing enteritis. [46,47,50] The histo-pathological changes and the course of GVHD have been studied in detail in the rat model. Shraut et. al. in 1986 studied GVHD in rats that had undergone both heterotopic and orthotopic small bowel transplantation. [47] A Lew rat was used for the donor and LBF-1 rat was used as the recipient. Like with Monchik & Russell's model the use of this combination in which the recipient is genetically incapable of rejecting the parenteral strain graft, an uninhibited GVH reaction would occur. They found that the first signs of GVHD appeared between the 9th and 11th day and consisted of redness, and swelling of the ears, snout, and paws and around the eyes. Over the next few days the skin became dry and scaly with some loss of hair. In the terminal stage between the 11th and 16th post-transplant day, the rats were emaciated, cold and listless and often sat on their hind legs. There was also an associated significant weight loss. These animals survived 14.4+/- 2.9 days in the heterotopic model, and 14.0 +/- 0.7 days in the orthotopic model. Autopsy findings included enlargement of the lymph nodes, and in some cases the spleen. In addition, an enteritis was noted that involved their own small bowel, whereas the allograft, whether orthotopic or heterotopic had a normal appearance.

Deltz's group in 1986 studying GVHD in the rat model attempted to find ways to circumvent this immunological phenomenon. They used a semiallogenic donor recipient combination by using Brown Norway donor rats and Brown-Norway-Lewis F1 hybrid recipients. A heterotopic intestinal transplant was performed in all animals which were then divided into the following groups: Group 1 received no treatment, Group 2 received

cyclosporine 15 mg/kg orally for 14 days, Group 3 received irradiation (950 rads), and Group 4 underwent microsurgical removal of their mesenteric lymph nodes. As expected all animals in group 1 died by 22 days post-transplant. Post-mortem examination revealed that the graft's mesenteric lymph nodes were greatly enlarged. Histological examination of these nodes showed evidence of maximum immunologic stimulation in the form of immunoblasts and macrophages. In addition it was also noted that the hosts peripheral lymph nodes as well as splenic tissue showed evidence of strong immunologic stimulation. In group 2, 71% of the animals survived until 150 days post-transplantation. It was postulated that the enlarged mesenteric lymph nodes in the graft represented the induction of GVHD in a donor compartment, while the immunologic stimulation of the host's peripheral lymph nodes and spleen represented the continuing proliferation of donor cells in the recipient. In groups 3 and 4, 77% and 89% of the animals respectively survived until 120 days. In these three groups (2, 3, and 4), there was no evidence of enlargement of the graft's mesenteric lymph nodes, nor was there any sign of immunologic stimulation of the hosts peripheral lymph nodes or spleen. This study infers that cyclosporine, sublethal irradiation of the donor, and most impressively removal of graft mesenteric lymph nodes are capable of circumventing the GVH reaction.[46]

Monitoring For Rejection

In order to perform a successful SIT one has to be able to monitor the allograft for any signs of rejection post-operatively. This applies to experimentation in the animal lab, as well as to clinical small intestinal transplantation. The small bowel allograft can be monitored both functionally and morphologically.

Functional Markers for Detection of Small Bowel Allograft Rejection

It would be ideal to have an inert marker or substrate whose absorption or lack thereof from the small bowel could determine whether the process of rejection was occurring.

This would provide a simple method for monitoring small bowel allografts for rejection without having to sample the bowel as in a mucosal biopsy or full thickness biopsy. Researchers have been experimenting with various substrates and inert markers which have proven promising in detecting rejection on the basis of absorption of these substances. As early as 1969, shortly after the introduction of small bowel transplantation, Cohen et. al. had begun to experiment with the absorption of carbon 14 labeled D-glucose and D-arabinose and attempted to determine the effect of rejection on the absorption of these substances.[51] They concluded that the loss of active transport of Carbon-14 labeled D-glucose by the small bowel allografts was a useful index for predicting impending rejection. Later in 1974, Stamford, working with the same group, studied fatty acid absorption in jejunal autografts and allografts.[52] They used mongrel dogs as a study animal, and studied the functional capability of jejunal autografts and allografts in relation to the absorption of short chain fatty acids (Lauric acid) and long chain fatty acids (Oleic acid). They found that the changes in Carbon-14 labeled Lauric acid absorption correlated closely with the histological appearance of the intestinal mucosa during rejection. They claimed to have been able to predict the prognosis of the allograft in terms of rejection by analyzing lauric acid absorption. Norgren et. al. investigated the intestinal absorption of cyclosporine and the uptake of glucose from the allografted small bowel and compared these parameters to graft histology. They found that the absorption of cyclosporine was significantly reduced when the small bowel underwent rejection. They also found that in the dogs where the allograft was undergoing rejection, there was a delayed appearance of peak uptake of carbon 14 labeled glucose and a significantly reduced absolute uptake. They concluded that the cyclosporine absorption and uptake of radiolabeled glucose can be utilized as functional monitors of intestinal allograft rejection.[53] Maltose absorption which requires an intact brush border for breakdown and absorption was evaluated as a marker for impending rejection of small intestinal allografts. It was shown in the rat model that impaired maltose absorption

predated the histological changes that occurred during rejection. One limitation of this test, however, was that a certain degree of mucosal damage was necessary before a discernible and measurable impairment of gut function occurred.[54] Due to Cr-EDTA's success in screening for intestinal permeability changes associated with a variety of inflammatory conditions of the small bowel, Grant and colleagues evaluated its use as a monitor for early rejection of intestinal allografts in the rat model. They found that initially a transient permeability defect occurred after intestinal grafting. However, once the graft had recovered from this injury, Cr-EDTA was a sensitive marker for intestinal rejection.[55] Since ^{99}Tc- DTPA had a shorter half-life than Cr-EDTA and was a common clinically used isotope, D'Alesandro and colleagues felt it would be better suited for measurement of intestinal permeability following clinical SIT. They found that ^{99}Tc-DTPA revealed an abnormal permeability study at least 48 hours prior to the onset of clinical symptoms or histological findings of rejection. It was thus felt that ^{99}Tc-DTPA may be a useful indicator of early rejection in SIT. [56]

Morphological Analysis for Rejection

Lillehei was the first to describe the histological appearance of small bowel rejection, when he was able to keep small bowel transplanted dogs alive for greater than three weeks. These classic histological signs of rejection include: mucosal bleeding, sloughing of the mucous membranes, disappearance of the villi, multiple ulcerations, perforation, thickening of the bowel wall, and perivascular cellular infiltrate in the submucosa.[5] These histological signs of rejection have been used as the gold standard over the past 30 years to confirm rejection of small bowel. The sequence of histological changes in acute and chronic rejection has been studied in detail in the rat model.[57] The process of rejection has been broken down into 3 phases. In acute rejection the allograft is normal up until day 6 (beginning of phase I) when lymphocytes and plasma cells begin to infiltrate the lamina propria. Phase II occurs on the 8th day when the cellular infiltrate

intensifies in the mucosa and submucosa and extends into the muscularis propria. This phase is also associated with shortening and blunting of the villi with some epithelial sloughing. The 10th day marks the beginning of phase III during which there is complete mucosal destruction, as well as heavy transmural cellular infiltration. In chronic rejection, the allograft is again normal up until day 6 when phase I starts and consists of intact villous architecture with lymphocyte and plasma cell infiltration. Phase II begins on the 10th day and consists of villous edema and blunting, scattered epithelial sloughing, and increasingly prominent cellular infiltrate. Phase III starts on the 13th day at which time there is destruction of mucosa and a transmural cellular infiltrate. The submucosa and muscularis are noted to be thickened and fibrosed but intact. At this point, the entire allograft coalesces and becomes encapsulated. It was noted that in the chronic model, because phase III was characterized by graft fibrosis, shrinkage and encapsulation, there was a poor correlation between the allograft's histological characteristics and the appearance of the recipients.

The obstacle to overcome was how to make use of these histological signs of rejection as monitors to assess the graft while the graft was still in situ. Several researchers devised methods of accessing the graft, like Monchik and Russell[49] who used a heterotopic graft with exteriorization of the proximal and distal ends as stomas, or Fortner[30] who developed the blind ended pouch made into a stoma which could be used for accessing samples of bowel mucosa for assessment of histology. If one were to gain access to the graft through either a proximal or distal stoma or through a blind ended pouch, then one would be obtaining mucosal biopsies as opposed to full thickness biopsies of the bowel wall. The ability to diagnose rejection based on mucosal biopsies alone has remained controversial. Langreher et. al. in 1992 studied graft morphology and serum chemistry profiles during chronic rejection of intestinal allografts in the rat. [58] They compared rats that had undergone syngeneic to allogeneic small bowel transplantation. Both groups received cyclosporine for a 4 week duration. Graft biopsies

and serum chemistry profiles in these two groups were obtained on post operative days 10, 30 and 50. In the syngeneic transplanted group histological examination of graft biopsies from post-operative days 10, 30 and 50 were consistent with normal bowel morphology. In the allogeneic group, graft biopsies from days 10 and 30 were considered to be normal, however on post-operative day 50 they described an obliterated arteriopathy and arteritis that was observed in the graft mesenteric vessels, whereas the mucosa showed only mild infiltration and a normal architecture. Serum chemistry markers from both groups at all times remained normal. This group felt from its data that chronic rejection of intestinal allografts presents histologically as a progressive infiltration of mesenteric vessels and not necessarily as primary mucosal disease. They concluded from this that mucosal biopsies and serum chemistry markers by themselves would not be reliable indicators of rejection of an intestinal allograft.

With the limited success recently of human intestinal transplantation, researchers have been able to study the accuracy of histologically monitoring human small bowel allografts using serial endoscopic biopsies. Nakamuru, Todo et. al. described their experience with 9 intestinal transplant recipients (8 small bowel + liver, 1 small bowel alone).[59] They found that when there was severe distortion of the mucosal architecture with accompanying villous blunting and focal ulcerations along with neutrophil plugging of capillaries, treatment of these patients with increased immunosuppression resulted in resolution of any clinical symptoms as well as normalization of the appearance of the small bowel mucosa. They concluded from this that monitoring of small bowel allografts via endoscopic biopsies could correctly identify rejection as a cause of graft damage following human small intestinal transplantation.

With the invention of monoclonal antibodies, it has become possible to identify the subpopulations of lymphocytes and macrophages present in the mucosa of biopsies obtained from small intestinal allografts. It was hypothesized that by identifying certain subpopulations present in the mucosa, one would be able to predict when an allograft

would be undergoing rejection. Various investigators have studied the immunohistochemical changes accompanying rejection. Their findings are as follows: Hurlburt et al. found that KI-M6, KI-M7 reactive macrophages in the lamina propria, as well as HLADR expression in the crypt epithelia accompanied rejection.[60] Goulet et. al. found that CD3, CD25 lymphocytes, KI-M6 macrophages in the lamina propria as well as HLADR antigens on crypt enterocytes occurred at the time of rejection.[61,62] Hansman et. al. showed that KI-M6 and KI-M7 macrophages were found in the submucosa as well as CD3 lymphocytes in the crypts.[63] All these studies were conducted in human small bowel transplant recipients. The importance of these studies involve the ability to predict impending rejection based on the appearance of these characteristic sub-populations of lymphocytes and macrophages present on mucosal biopsies obtained from an in situ small bowel allograft.

Hypothesis and Study Objectives

The original hypothesis of this study stated that small intestinal transplants between haploidentical donors and recipients offered a survival advantage over transplants between mismatched donors and recipients. Due to early deaths from immunological causes in the haploidentical group, this hypothesis was abandoned for a new one, which states that SIT between matched donors and recipients has a survival advantage over mismatched transplants. Secondary aims of this study include determining whether a 150 cm ileal allograft is capable of absorbing sufficient amount of nutrients to gain weight as well as sufficient amount of cyclosporine to maintain therapeutic levels. In addition functional assessment of surviving allografts was carried out by measuring intestinal permeability.

Methods

Outbred littermate pigs weighing 10-15 kg underwent Swine Leukocyte Antigen (SLA) typing of their DR locus by Polymerase Chain Reaction- Restriction Fragment Length Polymorphism analysis (PCR-RFLP). Animals were then selected as donor- recipient pairs as follows: SLA matched (M) (n=8), Haploidentical (H) (n=4), and SLA mismatched (MM) (n=8). All animals underwent a two-stage orthotopic segmental ileal intestinal transplant. The allograft consisted of the distal 150 cm of ileum. The allograft was initially placed heterotopically, with portocaval drainage. Two weeks later, the native bowel was resected, and the allograft placed orthotopically. Animals were followed for a six week study period, and then underwent intestinal permeability studies with ^{99}Tc-DTPA. Representative histological specimens were obtained at the time of final laparotomy (6 weeks), following which the animals were euthanized.

SLA Typing

Outbred littermate pigs weighing 8-10 kg were bled of 10 mLs of blood from the vena cava, and the blood placed into heparinized blood tubes. The blood was spun at 3000 rpm for 10 minutes, and then using a Pasteur pipette, the white cell layer as well as 1/3 of the packed cell layer was carefully removed and placed into a 15 mL corning tube. RPMI was added to bring the suspension up to 7 mL and was then mixed. Ficoll-Paque (7mL) was placed into a corning tube, and the cell suspension was carefully layered on the Ficoll-Paque. The suspension was spun at 1500 rpm for 30 minutes with no brake. The mononuclear cell layer at the interface between the media and the Ficoll-Paque was removed and placed into a second corning tube. RPMI (7 mL) was added to the cells and the suspension was spun at 1500 rpm for 5 minutes. The cells were placed in cryovials, and then packed on dry ice for shipment to Guelph, Ontario. The specimens were received in Guelph the following day and underwent Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR- RFLP) analysis utilizing three

primers for DRB and two restriction endonucleases (Msp 1, Rsa 1) according to the method of Shia, Lewin, and Schook.[64,65] The procedure required 1 1/2 - 2 weeks for completion, and the results were reported as a combination of Msp 1 and Rsa 1 digestion of the DNA.

Transplantation of Small Intestine

Donor Operation

Animals were premedicated with intramuscular ketamine and atropine, and were then anesthetized with 4% halothane, and maintained on 1% halothane for the duration of the case. A right external jugular line was placed for venous access. A laparotomy was performed. The spiral colon was identified, and the peritoneal attachments taken down so as to expose the mesentery of the colon. A point on the transverse colon immediately exiting the spiral colon was transected, following which the mesentery of the colon was divided to a point 10 cm proximal to the ileocecal valve. The ileum was divided with a GIA 50 stapler (autosuture), and the colon was then discarded. A pre-measured umbilical tape was used to measure a point 160 cm proximal to the distal resection margin of the ileum. The ileum at this point was stapled using a GIA 50 stapler. This 160 cm of ileum would become the allograft. The remaining ileum and jejunum were resected staying peripheral in the mesenteric arcade at the level of the vasa recta. In the pig, the duodenum and large bowel wrap around each other at the level of the pancreas, and both of these structures also wrap around the root of the small bowel mesentery containing the SMA, and SMV. It was therefore necessary to unravel the duodenum as well as the large bowel, to gain access to the superior mesenteric vessels. The portal vein was cleared to above the level of the splenic vein. The splenic vein was ligated adjacent to the portal vein, following which a splenectomy was performed to gain better access to the supraceliac aorta. The pancreas was peeled off the portal vein, and splenic vein and then removed. The infrarenal aorta was cannulated with a 10 Fr aortic

cannula. The supraceliac aorta was encircled with a ligature. The celiac artery was ligated just distal to the aorta. The animal received heparin 4000 units iv and phentolamine 1mg/kg iv. The graft was flushed in situ with 2 Litres of Ringers + 2.4% Mannitol solution. The portal vein was transected just above the splenic vein to vent the system. The allograft was removed with a large aortic cuff.

Recipient Operation

Stage I

The animal was anesthetized in the same manner as the donor. A double lumen catheter was placed in the right external jugular vein, and tunneled to the back of the animal. The animal received Cefazolin 500 mg iv, and Ranitidine 25 mg iv. A laparotomy was performed, and the small bowel and colon were reflected superiorly to visualize the infrarenal aorta and IVC. The infrarenal IVC was cleared for 4 cm, and the aorta was cleared for 3 cm using blunt dissection. This was often the most difficult part of the operation as there is a lymphatic sac that usually lies on the anterior aspect of the aorta, and must be gently pushed to the side so as not to enter it. If this sac is entered a lymphatic leak will result, and unless it is closed intraoperatively, the animal will develop postoperative ascites. Other researchers have described similar concerns with this lymphatic sac.[24] The animal received heparin 100 mg/kg iv, and then proximal and distal vascular clamps were placed on the IVC. A large venotomy was made in the IVC, and the donor portal vein anastomosed end-to-side using a running stitch of 6-0 polypropylene. Following completion of the venous anastomosis a bulldog clamp was placed on the donor portal vein while the vascular clamps on the native IVC were removed. We found it more effective to use a Satinsky clamp on the aorta rather than two vascular clamps as this was a safer way of dealing with the lymphatic sac. An arteriotomy was made, and then the superior part of the donor aortic cuff was anastomosed end-to-side to the recipient aorta using a running stitch of 6-0

polypropylene. Prior to releasing the Satinsky and bulldog clamps the animal was given sodium bicarbonate 3 mEq/kg, and calcium chloride 30 mg/kg. The grafted bowel immediately regained its natural pink color. The distal end of the allograft was brought out through a stoma in the right lower quadrant. The abdomen was closed, and the stoma matured. The animal received 1 litre of Ringers Lactate solution throughout the operation

Stage II (Performed two weeks following stage I)

The animal was anesthetized in the same manner as in stage I. A new external jugular line was placed on the left side, and tunneled to the back. The laparotomy incision was reopened, and the native bowel and allograft were dissected free from one another as there were usually many adhesions. The native bowel was marked off 10 cm proximal to the ileocecal valve, then all of the ileum and jejunum were resected proximally. The proximal end of the allograft was anastomosed to the duodenum. The distal end of the allograft was left as a stoma, but at 10 cm proximal to the stoma, the allograft was divided using a GIA 50 stapler, and the new distal end was anastomosed to the remaining native ileum. The original distal end of the allograft became a Thiry Vella Fistula. All anastomoses were constructed using 4-0 silk in interrupted stitches. The abdomen was closed. The animal received 750 mL of Ringers Lactate solution during the procedure.

Control Procedure

Animals were premedicated with intramuscular atropine and ketamine and were then anesthetized with 4% halothane until surgical anesthesia was attained at which point they were maintained on 1% halothane. A laparotomy was performed. The small bowel was identified, and a point 10 cm proximal to the ileocecal valve was marked. Using a pre-measured umbilical tape the small bowel was measured for 160 cm proximal to the distal marking. At this point the small bowel was divided using a GIA 50 stapler (autosuture). Next the bowel proximal to this point was resected staying distal in the mesentery. This

was carried out up to the point where the jejunum meets the c-loop of the duodenum, at which point the bowel was transected and discarded. Continuity was reestablished by anastomosing the ileum to the duodenum. However, first the proximal 10 cm of ileum was used to construct a Thiry Vella fistula. A sham transection and reanastomosis was carried out at the point marked previously 10 cm proximal to the ileocecal valve, and then the abdomen was closed, and the stoma matured.

Immunosuppression

All animals received methylprednisolone 1 mg/kg iv daily for 3 days post-op. Cyclosporine was given initially iv, and was subsequently changed on day 5 to oral cyclosporine. The initial starting dosages were 7 mg/kg/day iv, 20 mg/kg/day orally following stage I, and 30 mg/kg/day orally following stage II. Cyclosporine was dosed twice daily, and all doses were adjusted to maintain a target twelve hour whole blood trough level of 250-350 ng/mL.

Postoperative Care

All animals were housed initially in metabolic cages, and were allowed nothing by mouth for the first 48 hours. During this time they received 500 mL of iv D5 Saline + 20 mEq KCl/L twice a day. In addition, they received Cefazolin 500 mg iv, and Ranitidine 25 mg iv, each twice a day. Buprenorphine 0.4 mg iv twice daily was given for analgesia. These medications were given for the first 48 hours post-op. On post-op day 3, the animals were allowed water ad lib, and were then progressed to porcine mix the following day, and then started on pig chow on post-op day 5. They were then continued on pig chow and water ad lib until the end of the study period. In addition, all animals were supplemented with an elemental form of enteral nutrition (Vivonex).

Monitoring

a) Cyclosporine Levels

Whole blood cyclosporine levels were determined using the Abbott TDX monoclonal Radioimmunoassay. Levels were determined twice weekly for the first week, and subsequently weekly.

b) Weight Gain

Animals were weighed weekly. Weight gain was expressed as a percentage of the starting weight for the control animals. In the transplant group weight gain was expressed as a percentage of the pre-op stage II weight (i.e. reflecting the time that the allograft was in the orthotopic position)

c) General Well-Being

All animals were monitored daily for feed intake, excretions, and general well-being. Animals were observed daily for signs of GVHD (dermatitis, diarrhea, lymphadenopathy), or rejection (weight loss, diarrhea, decreased appetite, lethargy)

Determination of Study Endpoint

There were three possible ways that the study could be ended:

1. Animals that survived the 6 week study period were euthanized at the time of the final laparotomy.

2. At the time of the second stage procedure, if the allograft was determined to be nonviable by visual inspection, the animal was euthanized at that time.

3. If during any other time in the study the animal looked unwell including either weight loss > 10% of the starting weight, continued loss of appetite, persistent diarrhea, persistent vomiting, or persistent lethargy, then the animal was euthanized.

Obtaining, Processing, and Evaluation of Histological Specimens

Under any of the above circumstances, prior to euthanizing the animal the following full thickness specimens were obtained under anesthesia:

1. allograft
2. native small bowel
3. allograft mesenteric lymph nodes
4. native mesenteric lymph nodes
5. spleen
6. liver
7. skin

The biopsied tissue was fixed in 10% buffered formalin. Paraffin-embedded tissues were sectioned at 4 micrometers, and stained with hematoxylin and eosin. Biopsies were interpreted by a gastrointestinal pathologist.

Intestinal Permeability Testing with DTPA

During the sixth week of the study period, recipients of intestinal allografts underwent intestinal permeability studies. DTPA was supplied in a 1 mL volume containing 1000 MBq. This was diluted to 10mL creating 5 doses of 2mL, each dose containing 200MBq, which was equivalent to 5.41mCi. During transportation and preparation of the test solution one half life (6 hours) elapsed , and it was determined that each dose would now contain 2.71 mCi. Standard solutions were prepared by diluting one 2mL test solution to 1: 200 and a second test solution to 1: 500. Each pig to be studied was placed in a metabolic cage, and then gavaged with 2mL of test solution. The urine was collected after 7 hours and the volume measured. Three urine samples (1 mL each) of each animal were tested. Three samples of the prepared standard solutions were taken as well. Each urine sample was counted in a gamma counter along with one sample from each of the standard solutions to verify rate of decay. The average of the three samples was used. Percent recovery was calculated as:

$$\frac{\text{cpm/mL urine x volume of urine}}{\text{cpm/mL standard x dilution factor}}$$

Results were expressed as percent of injected dose.

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Means between the groups (control versus SLA matched versus SLA mismatched) were compared using an ANOVA. Duncan was used as the post-test. A p value <0.05 was considered statistically significant. Survival analysis was performed using Kaplan-Meier estimation as well as Cox proportional hazards survival analysis.

Results

Control Animals

Survival

One animal died of a technical complication (bowel obstruction) on post-op day #21. The remaining four animals (table 2.1) survived the study period.

Intestinal Permeability

The mean percent urinary recovery of Tc-DTPA was 0.18±0.08 (table 4.4)

Transplant Animals

SLA Matched(M)

Survival

In this group (table 4.1), One animal (T13) was lost due to a technical complication (closed loop bowel obstruction) at day 7. Three animals T12, T17 and T19 were euthanized at days 24, 27 and 10 respectively for clinical evidence of rejection that was confirmed histologically. One animal (T18) was euthanized at day 9 for histological evidence of GVHD. The remaining three animals (T4, T14, T21) survived the study period.

Histology

Histological samples from the allograft of T12 showed that the mucosa was ulcerated and had sloughed. In addition there was an infiltrate of inflammatory cells extending from the remaining mucosa down to the muscularis propria. The allograft mesenteric lymph node showed evidence of its architecture being effaced with areas of necrosis surrounded by reactive fibrosis. The mucosa of T17's allograft was sloughed in areas and there was an infiltrate of lymphocytes in the submucosa and in a

perivascular distribution within the muscularis propria. The histology of T19 revealed that the mucosa of the allograft had sloughed in areas with an infiltrate of mononuclear cells. T18 showed histological evidence of GVHD in several tissues (table 4.5) including: karyorrhectic nuclei in the crypt epithelium of the native jejunum, as well as an infiltrate of plasma cells and immunoblasts in the native and grafted mesenteric lymph nodes and spleen. The bile ducts of the liver were surrounded by lymphocytes with an associated endothelialitis. The histological findings were consistent with acute rejection in T12, T17, and T19, and with GVHD in T18. No evidence of rejection or GVHD was evident in either T4, T14, or T21.

Intestinal Permeability

The mean percent recovery of Tc-DTPA was 0.15±0.12 % (table 4.4).

SLA Haploidentical (H)

Survival

Excluding one technical death (T22-due to bowel obstruction) on day 13, haploidentical transplants (table 4.2), survived a mean of 15 days. Two animals showed signs of GVHD (dermatitis, diarrhea, lymphadenopathy). One animal developed severe rejection. None of these animals survived long enough to undergo the second stage procedure.

Histology

Transplant animal T5 showed histological evidence of GVHD in several tissues (table 4.5) including: blunted villi and karyorrhectic nuclei in the crypts of the native jejunum, an infiltrate of plasma cells and immunoblasts in the allograft mesenteric lymph node, as well as an infiltrate of plasma cells and immunoblasts in the native mesenteric nodes. In addition there was an infiltrate of immunoblasts and plasma cells

found in the spleen. The bile ducts of the liver were surrounded by lymphocytes with an associated endothelialitis. The skin showed dyskaryotic cells in the epidermis and a mononuclear cell infiltrate in the upper dermis. Histological sampling from T6 also revealed evidence of GVHD (table 4.5) including: congestion of the villus tips in the native jejunum, an infiltrate of immunoblasts in the allograft mesenteric lymph node as well as the native mesenteric lymph node, and the spleen. The skin showed scattered necrotic nuclei. Histology from the allograft of T7 showed sloughing of the mucosa, and an infiltrate of lymphocytes and eosinophils extending from the superficial submucosa into the muscularis propria. The allograft mesenteric lymph nodes showed necrosis with acute inflammation and surrounding reactive fibrosis. These findings in T7 were consistent with acute rejection (table 4.5).

SLA Mismatched (MM)

Survival

In this group (table 4.3), there was one technical death due to thrombosis of the venous anastomosis (T16). Four animals T9, T11, T20, T23 were euthanized due to clinical evidence of rejection at days 14, 24, 14, and 13 respectively. This was confirmed histologically. Three animals (T10, T15,T24) survived the study period.

Histology

Histological samples from the allograft of T9 showed marked villus blunting as well as an infiltrate consisting of neutrophils, eosinophils, and lymphocytes extending from the mucosa down to the muscularis propria. In addition the allograft mesenteric lymph nodes were effaced with areas of necrosis surrounded by reactive fibrosis and lymphocytic activation. Histology from the allograft of T11 revealed villus blunting as well as edema of the villus tips in association with an infiltrate of lymphocytes and eosinophils extending from the lamina propria down to the muscularis propria. In

addition the sinuses of the mesenteric lymph nodes were expanded and contained increased number of lymphocytes neutrophils and eosinophils. T20's allograft showed mucosal sloughing with an infiltrate of lymphocytes within the submucosa and muscularis propria. The histology of T23's allograft revealed mucosal sloughing in some areas with an infiltrate of mononuclear cells in the mucosa, submucosa, and in a perivascular distribution in the bowel wall. The histological findings in T9, T11, T20, and T23 (table 4.5) were consistent with acute rejection. No histological evidence of rejection or GVHD was observed in T10, T15 or T24.

Intestinal Permeability

The mean percent urinary recovery of Tc-DTPA was 3.22±2.68 (table 4.4)

Discussion

There does not appear to be a significant survival advantage for SLA matched transplants over mismatched transplants (figure 4.1). This is in disagreement to the work of Meijesen who showed that there was a survival advantage to using DLA matched transplants over DLA mismatched transplants in canine SIT.[66] Even though our pigs were only matched at the class II locus, the fact that they were littermates makes it unlikely that they would be completely mismatched at the class I locus.[67] Furthermore, even if they were completely mismatched at Class I, Sachs group working in miniature swine have shown that recipients of kidney allografts that are matched at Class II but mismatched completely at Class I develop tolerance to the graft when cyclosporine is given for the first 12 days.[68] This would suggest that our animals that are matched at class II and receiving cyclosporine therapy would have a survival advantage over those mismatched at Class II. It is likely that the increased immunogenicity associated with an SIT makes it unable to share the same advantage as a matched kidney allograft. Immunologically, the mismatched animals tended to be susceptible to rejection, with 4 of 7 animals succumbing to rejection during the study period. There was no evidence of GVHD in the mismatched animals. The matched animals, however, were susceptible to both GVHD and rejection, with one animal dying from the former and three from the latter. Excluding technical failures 35% of transplanted animals survived the study period. The target level for CsA was low in our study animals which ordinarily has been shown not to be consistent with survival in pig SIT.[22] Other researchers have shown that segmental SIT is possible with lower levels of CsA but these were still somewhat higher than ours.[26] It leads one to believe what has always been assumed, that there is a possible survival advantage in littermate SIT vs. non littermate SIT. This would need to be proven in a proper study actually comparing the two, however, it may have implications for living related SIT.

With the partial protection from rejection that littermate animals may provide comes an obstacle in the form of GVHD. In our series of transplant animals there were three cases of GVHD (table 4.4). Two of these cases occurred in the haploidentical transplants, and one in the matched group. This phenomenon was not seen in the mismatched animals. GVHD presented by day 8 post-op stage I as a dermatitis involving the thorax and abdomen. Over the next week the dermatitis progressed to become more extensive involving the abdomen and thorax, and also now involved the ears. In addition, during this week, the animals developed diarrhea, and in one case lymphadenopathy. These animals lost weight and became cachectic. All animals followed the same downward course, and by two weeks post-transplant (stage I), all animals were euthanized due to weight loss, cachexia, unwillingness to eat, and persistent diarrhea.

The search for histological evidence of GVHD encompasses many organs. Unlike rejection of small intestinal allografts which involves primarily donor tissue, GVHD effects both donor and recipient tissue. All animals that exhibited GVHD in our study showed characteristic findings in both the native and donor mesenteric lymph nodes, as well as the recipient's spleen. The characteristic cell the immunoblast[50,69], was evident in large numbers in all three lymphatic compartments. The immunoblast which is presumably stimulated in the grafted mesenteric lymph node migrates to recipient mesenteric lymph nodes and spleen. This process has been shown not to be random, but to be mediated by specific interactions between lymphocytes and the endothelial cells lining postcapillary high endothelial vessels of the spleen, and mesenteric lymph nodes.[70] The verification that these lymphocytes seen in the spleen and mesenteric lymph nodes of the recipient are of donor origin has been carried out by Murase et al utilizing monoclonal antibodies specific for donor and recipient lymphocytes.[71] Histologically T5 and T18 appear to have undergone a more severe GVH reaction than T6. This is evident in three areas: Firstly, the skin shows the characteristic karyohexis with an infiltrate of lymphocytes at the basal layer of the epidermis. Secondly, lymphocytes are seen

surrounding the bile ducts in the liver with an associated endothelialitis. This has been shown in the rat model to be consistent with a severe GVH reaction.[47] Thirdly, and most importantly, the native intestine shows the presence of karyorhectic cells in the epithelium of crypts as well as acute inflammation of the mucosa with blunting of the villi, which could be consistent with an enteritis. We think this enteritis was likely the cause of death in these animals. Deltz hypothesized that due to the spectrum of histological changes observed in GVHD, animals would be capable of surviving the infiltration of lymphocytes in the recipient lymphoid tissue, whereas the associated enteritis would result in a lethal outcome. [50]

It is unclear why lethal GVHD was observed in our study animals. According to Deltz, the strength of a GVH reaction is closely correlated to the quantity of lymphatic tissue grafted.[50] Based on these findings one would expect that the allograft in our study representing 20% of the small bowel in length would be less likely to be involved in a severe GVH reaction. However, the severity of the GVH reaction seen in our segmental ileal grafts was much more severe than models utilizing the entire length of the small bowel.[29] There are two theories that may explain the basis for this phenomenon. Kizilisik and colleagues have shown in the rat model that retention of the native small intestine in the heterotopic intestinal transplant model significantly increases the severity of GVHD following transplantation.[69] In our study, pigs underwent a two stage segmental SIT in which the allograft was initially placed heterotopically. In this way the native bowel was retained over a period of two weeks. It is possible that this retained bowel with its large amount of GALT increases the likelihood of a GVH reaction. However, since all animals (matched, haploidentical, mismatched,) had a heterotopic graft initially, and only the haploidentical and matched groups showed evidence of GVHD, the retained native bowel would be unlikely to be the only reason for the severe GVH reactions. The second theory would involve the possibility that haploidentical and matched transplants can create a semiallogenic process similar to the one-way parent-to-

F1 hybrid model developed by Monchik and Russell.[49] If these animals are incapable of rejecting the graft, but the graft is capable of rejecting the host, then a GVH reaction may ensue. It is possible that it is the combination of these two theories that increases the likelihood of GVHD. A predisposition towards GVHD brought about by the partial matching coupled with the large amount of GALT present in retained small bowel may explain the findings of a lethal GVH reaction.

In human clinical SIT, these findings are of extreme importance. One would expect that a SIT from parent to child would be the most likely form of living related SIT for an infant with short bowel syndrome. The parent - child combination provides for a haploidentical transplant. Our findings in the pig model suggest that there may be a trend towards increased risk of GVHD in this group versus the mismatched. If this is the case, then careful consideration must be given to parent - child transplantation especially in situations where there may be retention of native small bowel such as in pseudo-obstruction syndrome. Perhaps in these situations, it may be necessary to monitor the patient more closely for early signs of GVHD and treat aggressively. Alternatively, it may prove beneficial to resect as much nonfunctioning native small bowel as possible thus reducing the amount of GALT.

The weight gain of the transplant animals was less than the controls but not significantly (figure 4.2). The transplant animals had to contend with two obstacles that the controls did not, that being the more stressful transplant procedure , as well as the regeneration of lymphatics. Despite this, transplant animals were able to gain weight , with all but one animal regaining their preoperative weight by 4 weeks following orthotopic placement of the allograft. Kimura's recipients of segmental jejunal allografts required 50 days to regain their preoperative weight.[26]

The ability of a short segmental intestinal allograft to provide for increasing nutrient absorption and weight gain relies heavily on the ability of that segment to adapt. Efforts were made to assist with this adaptation response in two ways. Firstly an ileal segment

was used which has been shown to have superior adaptive capabilities compared to jejunal segments. [72] Secondly, all animals were supplemented with Vivonex. In addition to the beneficial effect an elemental diet has in aiding the adaptation response of a short segment of bowel,[72] this compound contains glutamine which has also been shown to be beneficial in the adaptation response.[73]

All transplant animals were able to maintain the target cyclosporine levels (table 4.4). The importance of this is that the 150 cm segmental ileal allograft was capable of absorbing sufficient amounts of orally administered cyclosporine to maintain therapeutic levels.

In the surviving transplant animals, all had histologically normal appearing allografts as well as native intestine. Functional assessment of these allografts were carried out by measuring intestinal permeability with DTPA (table 4.4). Due to its short half-life and the fact that it is a common clinically used isotope, DTPA has become a useful substance for measuring intestinal permeability. An increased intestinal permeability was evident in the mismatched transplants. Measurement of intestinal permeability has been shown to be a valuable marker for monitoring intestinal allografts for rejection. Permeability has been shown clinically and experimentally to increase with rejection.[55,56,74] D'Alesandro and colleagues have shown in a very small series of patients that DTPA was useful for indicating early rejection of intestinal allografts.[56] Perhaps this increased permeability seen only in the mismatched group represents an early rejection pattern that is only manifesting functionally and not morphologically at this point. This would suggest that although this study did not demonstrate a survival advantage for matched allografts over mismatched allografts in the short term (6 weeks), a longer study period may have.

There are several areas that require further study. The surgical technique requires a modification if it is to be compatible with survival of the donor in a living related SIT. This would require isolation of the SMA at a more peripheral location so as to leave the aorta and the proximal SMA intact. Increased intestinal permeability was evident in the

mismatched group. If this does represent an early rejection pattern, then more frequent DTPA measurements over a longer study period would provide valuable data to make a final conclusion in this matter. In addition the use of monoclonal antibodies to identify lymphocytes that are present in the allograft during early rejection may further validate the use of intestinal permeability as a marker for early rejection. We believe that the combination of partial matching of donor and recipient as well as initial heterotopic placement of the graft increases the risk of GVHD. In order to prove this conclusively it would be necessary to perform both heterotopic and orthotopic transplants in two separate groups with each group being divided into matched and mismatched transplants. This would show whether matching alone, heterotopic placement of the bowel alone, or a combination of both increases the risk of GVHD.

Table 4.1 Results - SLA Matched Transplants

Animal I.D.	Technical loss	GVHD	Rejection	CsA level (ng/mL)	Survival (days)	Wt. Gain (%)
T4	no	no	no	338.98±260.38	40	9.71
T12	no	no	yes	200.23±271.88	24	N/A
T13	yes	no	no	N/A	7	N/A
T14	no	no	no	299.92±364.19	40	-2.94
T17	no	no	yes	332.94±100.25	27	N/A
T18	no	yes	no	403.3±334.60	9	N/A
T19	no	no	yes	419.66±286.01	10	N/A
T21	no	no	no	532.68±233.92	40	25

Table 4.2 Results - Haploidentical Transplants

Animal I.D.	Technical loss	GVHD	Rejection	CsA level (ng/mL)	Survival (days)	Wt. Gain (%)
T5	no	yes	no	296.94±127.78	17	5.63*
T6	no	yes	no	412.43±95.09	14	-9.48*
T7	no	no	yes	589.06±285.32	14	3.45*
T22	yes	no	no	N/A	13	N/A

* allograft in heterotopic position, second stage not performed

Table 4.3 Results - SLA Mismatched Transplants

Animal I.D.	Technical loss	GVHD	Rejection	CsA level (ng/mL)	Survival (days)	Wt. Gain (%)
T9	no	no	yes	342.75±124.88	14	N/A
T10	no	no	no	259.11±160.89	40	17.78
T11	no	no	yes	251.58±278.83	24	N/A
T15	no	no	no	439.63±236.11	40	-1.32
T16	yes	no	no	N/A	2	N/A
T20	no	no	yes	349.7±238.27	14	N/A
T23	no	no	yes	369.2 *	13	N/A
T24	no	no	no	377.4±242.26	40	42.6

* only one cyclosporine level taken

Table 4.4 Summary - Transplant Animals

Group	n	Tech-nical loss	GVHD	Rejection	CsA level (ng/mL)	Sur-vival (n)	Wt Gain (%)	DTPA (% recovery)
Control	5	1	0	0	208.49± 50.87	4	20.19±20.54	0.18±0.08
M	8	1	1	3	361.10±104.53	3	12.06±11.93	0.15±0.12
H	4	1	2	1	432.81± 147.12	0	N/A	N/A
MM	8	1	0	4	341.33± 66.62	3	15.26±15.46	3.22±2.68 *

* $p<0.05$ compared to M using ANOVA

Table 4.5 Histology Summary

Animal I.D.	T4	T12	T14	T17	T18	T19	T21	T5	T6	T7	T9	T10	T11	T15	T20	T23	T24
Allograft	-	+	-	+	-	+	-	-	-	+	+	-	+	-	+	+	-
Native Jejunum	-	-	-	-	*	-	-	*	*	-	-	-	-	-	-	-	-
Allograft Mesenteric Lymph Node	-	+	-	-	*	+	-	*	*	+	+	-	+	-	-	+	-
Native Mesenteric Lymph Node	-	-	-	-	*	-	-	*	*	-	-	-	-	-	-	-	-
Spleen	-	-	-	-	*	-	-	*	*	-	-	-	-	-	-	-	-
Liver	-	-	-	-	*	-	-	*	-	-	-	-	-	-	-	-	-
Skin	-	-	-	-	*	-	-	*	*	-	-	-	-	-	-	-	-

+ Rejection * GVHD - no evidence of Rejection or GVHD

Figure 4.1 **Pig S.I.T. Survival**

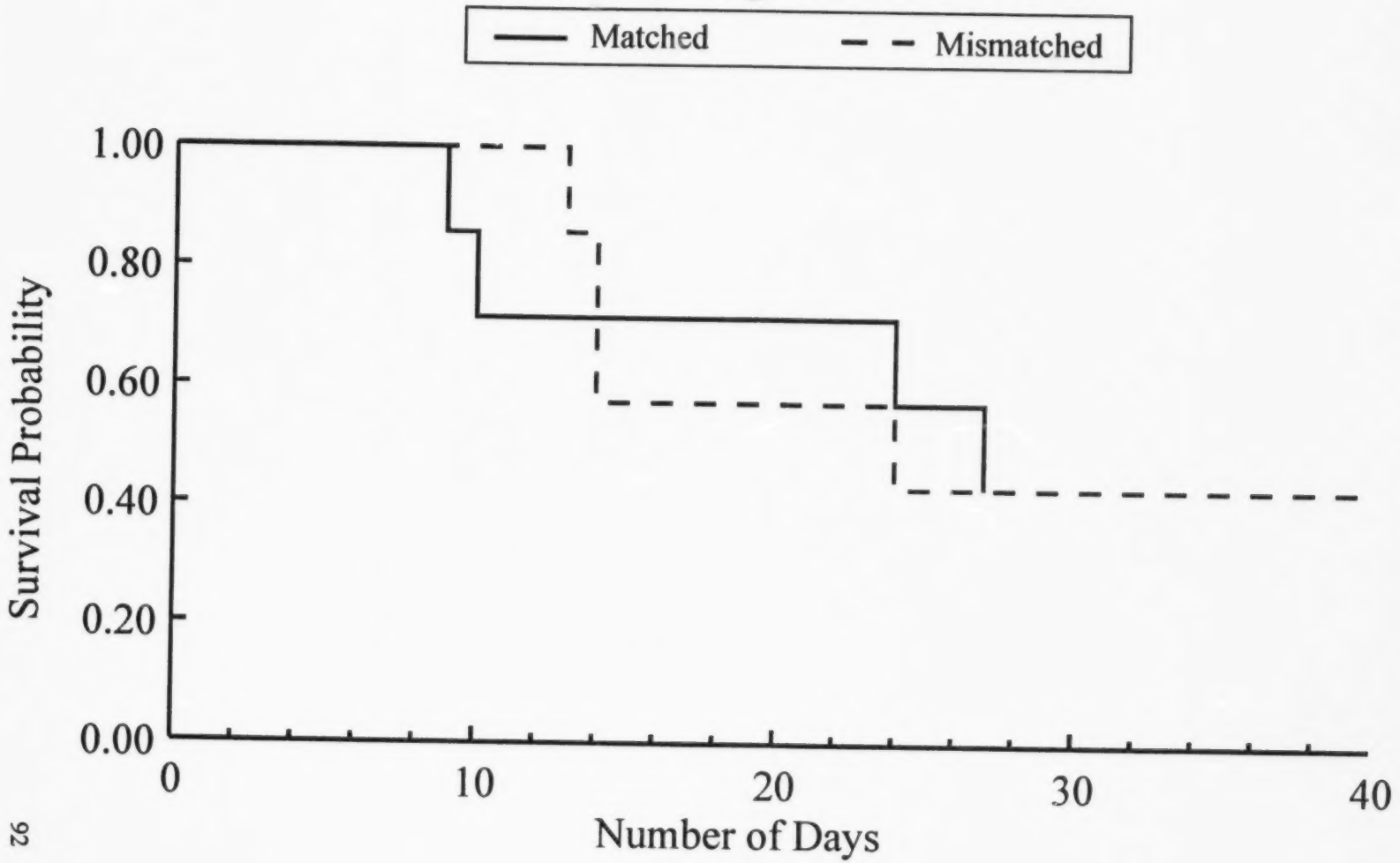
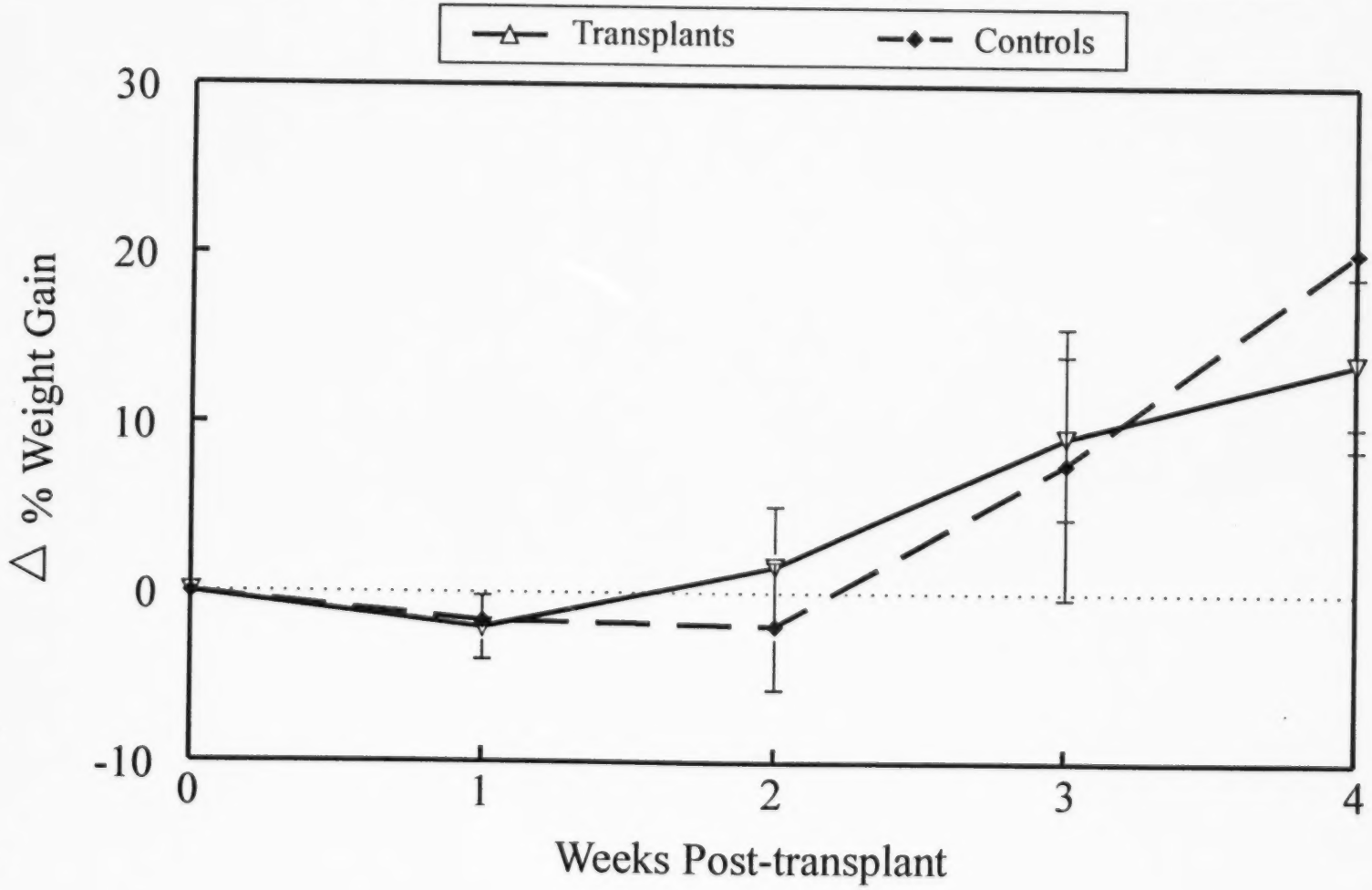


Figure 4.2 **Weight Gain: Transplants vs Controls**



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Conclusion

Although research in the field of small intestinal transplantation (SIT) has been carried out for over 30 years, this procedure is still considered experimental with only 6 centres in the world performing isolated SIT.[1,2,3,4,5,6,7,8] Due to the large amount of lymphoid tissue present in the intestine, this organ is more immunogenic than almost any other organ with the possible exception of bone marrow and spleen.[9,10,11] The main obstacle to success in this procedure is a lack of proper balance of immunosuppression, with insufficient amounts resulting in rejection, and appropriate amounts increasing the risk of sepsis, and lymphoproliferative disease.

Long term survival of renal allografts has been shown to be possible by obtaining HLA matched living related donors.[12,13,14] It was hypothesized that this form of immunomodulation may afford intestinal allografts with a survival advantage. In order to study this in detail we have developed a new animal model for segmental ileal intestinal transplantation.

The pig was chosen as the study animal due to its similarities to humans anatomically, physiologically,[15] and immunologically.[16] Swine Leukocyte Antigen (SLA) typing of donor and recipient is now possible for transplantation in outbred pigs. This can be carried out rapidly, with small quantities of blood utilizing PCR-RFLP analysis.[17,18] Due to the similarities between swine and human MHC loci, pig related transplantation utilizing SLA typing should be analogous to human related transplants with HLA typing.

An ileal allograft was selected due to its superior absorptive capacity, as well as its distinctive vascular supply. A segment of intestine measuring 20% of the total length was felt to be the minimum amount that a recipient could survive on.[19] We have shown that in the pig model recipients of segmental ileal intestinal allografts are capable of sufficient weight gain and cyclosporine absorption. This procedure is reproducible with a low (15%) technical failure rate. Considering the fact that large animal models of SIT have

been plagued with a high technical failure rate in the past, this model may be very useful for exploring other forms of immunomodulation (such as new immunosuppressants) of SIT in addition to living related transplants.

This study shows that transplantation of only the distal ileum can result in adequate absorptive capacity to allow weight gain, and adequate absorption of enterally administered cyclosporine to give therapeutic levels. There does not appear to be a survival advantage to matching of the SLA DR locus, however there appears to be increased intestinal permeability in the mismatched group. This may be due to an early rejection pattern that is not yet evident histologically.[20, 21] Unexpectedly some partially matched animals developed a lethal form of GVHD. It is believed that the combination of partial matching and initial heterotopic placement of the graft was responsible for the development of lethal GVHD. These could have implications for intestinal transplants in humans since partial matching or retention of native intestine (such as in pseudo-obstruction syndrome) may potentiate the development of GVHD.

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Appendix 1

Histology Results - T12

	SLA MATCHED	
ANIMAL ID #	**T12**	
ALLOGRAFT	mucosa ulcerated and has sloughed. Infiltrated by inflammatory cells from remaining mucosa down to mucularis propria.	
NATIVE JEJUNUM	mucosa mildly inflamed	
ALLOGRAFT MESENTERIC LYMPH NODE	lymph node architecture effaced. Areas of necrosis surrounded by reactive fibrosis	
NATIVE MESENTERIC LYMPH NODE	mild reactive changes	
SPLEEN	normal	
LIVER	normal	
SKIN	normal	
HISTOLOGICAL DIAGNOSIS	Acute Rejection	

Appendix 1 (continued)

Histology Results - T4, T14

SLA MATCHED

ANIMAL ID #	T4	T14
ALLOGRAFT	mild increase in number of eosinophils within lamina propria	mild increase in number of mononuclear cells in lamina propria
NATIVE JEJUNUM	normal	normal
ALLOGRAFT MESENTERIC LYMPH NODE	normal	normal
NATIVE MESENTERIC LYMPH NODE	N/A	mild reactive changes
SPLEEN	normal	normal
LIVER	normal	normal
SKIN	normal	normal
HISTOLOGICAL DIAGNOSIS	No evidence of Rejection or GVHD	No evidence of Rejection or GVHD

Appendix 1 (continued)

Histology Results - T17, T19

SLA MATCHED

ANIMAL ID #	T17	T19
ALLOGRAFT	mucosa sloughed in areas. Infiltrate of lymphocytes in submucosa and in perivascular distribution within muscularis propria	mucosa sloughed in areas. Infiltrate of mononuclear cells
NATIVE JEJUNUM	normal	normal
ALLOGRAFT MESENTERIC LYMPH NODE	reactive changes	zonal necrosis and vascular congestion
NATIVE MESENTERIC LYMPH NODE	mild reactive changes	reactive changes
SPLEEN	normal	normal
LIVER	normal	normal
SKIN	normal	normal
HISTOLOGICAL DIAGNOSIS	Acute Rejection	Acute Rejection

Appendix 1 (continued)

Histology Results - T18, T21

SLA MATCHED

ANIMAL ID #	T18	T21
ALLOGRAFT	normal	normal
NATIVE JEJUNUM	karyorrhectic cells in crypt epithelium	normal
ALLOGRAFT MESENTERIC LYMPH NODE	infiltrate of plasma cells and immunoblasts	mild reactive changes
NATIVE MESENTERIC LYMPH NODE	infiltrate of plasma cells and immunoblasts	normal
SPLEEN	infiltrate of plasma cells and immunoblasts	normal
LIVER	bile ducts surrounded by lymphocytes, endothelialitis	normal
SKIN	apoptotic cells seen within epidermis associated with lymphocytes. Lymphocytes and plasma cells in a perivascular distribution within upper dermis	normal
HISTOLOGICAL DIAGNOSIS	Graft Versus Host Disease	No evidence of Rejection or GVHD

Appendix 1 (continued)

Histology Results - T5, T6

HAPLOIDENTICAL

ANIMAL ID #	T5	T6
ALLOGRAFT	infiltrate in lamina propria consisting of lymphocytes, plasma cells, and eosinophils	normal
NATIVE JEJUNUM	karyorrhectic nuclei in the crypts, blunted villi, mucosa inflamed	congestion of villus tips
ALLOGRAFT MESENTERIC LYMPH NODE	infiltrate of plasma cells and immunoblasts	node enlarged, architecture effaced in areas, infiltrate of immunoblasts
NATIVE MESENTERIC LYMPH NODE	infiltrate of plasma cells and immunoblasts	infiltrate of immunoblasts and plasma cells
SPLEEN	infiltrate of plasma cells and immunoblasts	infiltrate of immunoblasts
LIVER	bile ducts surrounded by lymphocytes, endothelialitis	normal
SKIN	dyskaryotic cells in epidermis, mononuclear cell infiltrate in upper dermis	scattered necrotic nuclei
HISTOLOGICAL DIAGNOSIS	Graft Versus Host Disease	Graft Versus Host Disease

Appendix 1 (continued)

Histology Results - T7

	HAPLOIDENTICAL	
ANIMAL ID #	**T7**	
ALLOGRAFT	mucosa sloughed, superficial submucosa and muscularis propria infiltrated by lymphocytes, and eosinophils	
NATIVE JEJUNUM	normal	
ALLOGRAFT MESENTERIC LYMPH NODE	extensive areas of necrosis with acute inflammation and surrounding reactive fibrosis	
NATIVE MESENTERIC LYMPH NODE	reactive	
SPLEEN	normal	
LIVER	normal	
SKIN	normal	
HISTOLOGICAL DIAGNOSIS	Acute Rejection	

Appendix 1 (continued)

Histology Results - T9, T11

SLA MISMATCHED

ANIMAL ID #	T9	T11
ALLOGRAFT	marked villous blunting infiltrate of neutrophils, eosinophils, and lymphocytes in mucosa down to muscularis propria	villous blunting villous tips edematous infiltrate of lymphocytes, eosinophils from lamina propria down to muscularis propria
NATIVE JEJUNUM	infiltrate of chronic inflammatory cells in lamina propria	mild increase in number of inflammatory cells within lamina propria
ALLOGRAFT MESENTERIC LYMPH NODE	lymph node effaced areas of necrosis surrounded by reactive fibrosis and lymphocytic activation	sinuses expanded and contain increased number of lymphocytes, neutrophils and eosinophils
NATIVE MESENTERIC LYMPH NODE	mild reactive changes	reactive changes
SPLEEN	normal	normal
LIVER	normal	normal
SKIN	normal	normal
HISTOLOGICAL DIAGNOSIS	Acute Rejection	Acute Rejection

Histology Results - T10, T15

	SLA MISMATCHED	
ANIMAL ID #	**T10**	**T15**
ALLOGRAFT	normal	mild increase in the number of mononuclear inflammatory cells within lamina propria
NATIVE JEJUNUM	mild increase in number of inflammatory cells in lamina propria	mild increase in number of inflammatory cells in lamina propria
ALLOGRAFT MESENTERIC LYMPH NODE	mild reactive changes	normal
NATIVE MESENTERIC LYMPH NODE	mild reactive changes	normal
SPLEEN	normal	normal
LIVER	N/A	normal
SKIN	N/A	normal
HISTOLOGICAL DIAGNOSIS	No evidence of Rejection or GVHD	No evidence of Rejection or GVHD

Appendix 1 (continued)

Histology Results - T20, T23

	SLA MISMATCHED	
ANIMAL ID #	**T20**	**T23**
ALLOGRAFT	mucosa sloughed. Infiltrate of lymphocytes within submucosa and muscularis propria	mucosa sloughed in some areas. Infiltrate of mononuclear cells in mucosa, submucosa and in a perivascular distribution in the bowel wall
NATIVE JEJUNUM	normal	normal
ALLOGRAFT MESENTERIC LYMPH NODE	reactive changes	zonal necrosis with reactive fibrosis
NATIVE MESENTERIC LYMPH NODE	reactive changes	reactive changes
SPLEEN	normal	normal
LIVER	normal	normal
SKIN	normal	normal
HISTOLOGICAL DIAGNOSIS	Acute Rejection	Acute Rejection

Appendix 1 (continued)

Histology Results - T24

	SLA MISMATCHED	
ANIMAL ID #	**T24**	
ALLOGRAFT	mucosa mildly hypercellular	
NATIVE JEJUNUM	normal	
ALLOGRAFT MESENTERIC LYMPH NODE	mild reactive changes	
NATIVE MESENTERIC LYMPH NODE	mild reactive changes	
SPLEEN	normal	
LIVER	normal	
SKIN	normal	
HISTOLOGICAL DIAGNOSIS	No evidence of Rejection or GVHD	